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This report reviews the potential environmental hazard from the commercial use of 2-mercaptobenzothiazoles (MBT). Most of the mercaptobenzothiazoles are consumed as rubber accelerators in vulcanization processes, although the sodium salt of MBT is used as a corrosion inhibitor in water-based cooling systems. Information on physical and chemical properties, production methods and quantities, commercial uses and factors affecting environmental contamination, as well as information related to health and biological effects, are reviewed.

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MERCAPTOBENZOTHIAZOLES

Joseph Santodonato
Leslie N. Davis
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Jitendra Saxena

June 1976
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Project Officer
Frank J. Letkiewicz

Prepared for:
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Executive Summary

Mercaptobenzothiazole (MBT) compounds are produced in over 100 million pounds annually in the United States. They are mostly consumed as rubber accelerators in vulcanization processes, although almost 6 million pounds per year of the sodium salt of MBT are used as a corrosion inhibitor in water-based cooling systems (e.g., automotive cooling systems). The available information suggests that sizable quantities of MBT are being released to the environment from discarded coolants and rubber products, as well as from particles worn from tires. No information on the persistence of MBT compounds and no experimental data on bioaccumulation are available. However, physical properties of MBT compounds would suggest a low bioaccumulation potential. Some derivatives have been detected in water but rarely have concentrations been reported. The principal actions of MBT derivatives in human and animal systems include: (1) production of allergic contact dermatitis, (2) action on the central nervous system, and (3) inhibition of certain metalloenzymes which contain copper. The minimum levels to produce these physiologic actions have not been published. Furthermore, the potential for chronic exposure at sublethal doses to produce irreversible damage has not been investigated.

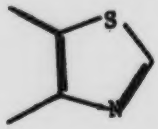
Several of the compounds have been screened for carcinogenic potential by long-term feeding to mice with negative results. However, mutagenesis assays in fruit flies suggest that several MBT compounds may be mutagenic (because of the deficiency of determining mutagenesis with fruit flies, these compounds should be tested with the Ames or similar tests). Although MBT compounds have a high potential for entering the environment, the exact amount of exposure to biological organisms and the risk involved from such exposure is difficult to estimate, due to a lack of information on environmental fate and toxicological effects at possible environmental concentrations. More detailed monitoring surveys and studies of the environmental fate of MBT would seem desirable.

I. Physical and Chemical Data

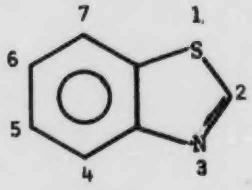
A. Structure and Properties

1. Chemical Structure

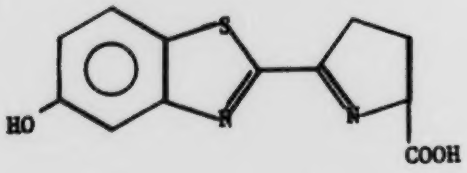
The thiazole ring is a five-member unsaturated aromatic system containing nitrogen and sulfur:



When the two adjacent carbons in a thiazole ring are also adjacent carbons of a benzene ring, the compound is called benzothiazole:

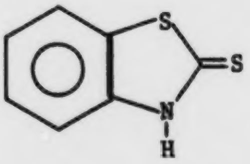


The S is defined as atom #1 in the benzothiazole ring. Substituent carrying atoms are numbered clockwise around the rings, as shown above (Allen, 1966). Although benzothiazole derivatives with substituents on the benzene ring are known, they are not of general commercial significance. Some of these derivatives occur in nature, for example, luciferin,

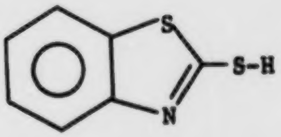


the enzymatic oxidation of which produces the characteristic luminescence of the firefly (Roberts and Caserio, 1965). The most important commercial derivatives are compounds in which the thiazole ring hydrogen has been replaced with a mercapto group or other thio derivatives.

2-Mercaptobenzothiazole (MBT) is a bicyclic polyhetero aromatic compound which exists in thioketo and thioenol canonical forms (Malik and Rahmani, 1975).



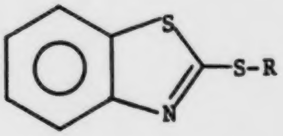
thioketo-MBT



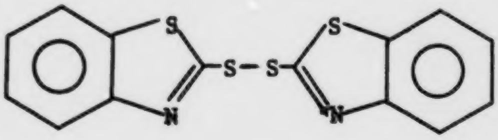
thioenol-MBT

Crystal structure studies show the molecule is planar and in the thioketo form in the solid state (Chesick and Donohue, 1971). Infrared spectral studies confirm the presence of the -N-C=S group in the solid compound, but metal complexes show evidence of coordination through the mercapto sulfur exclusively, indicating conversion of thioketo-MBT to thioenol-MBT on reaction with metals (Khan and Malik, 1972). The absence of an N-H bond as well as a metal-N bond has been demonstrated by IR studies on compounds of MBT with Cu(II), Ni(II), Co(II), Cd(II), Zn(II), Pb(II), Ag(I), and Tl(I) (Khullar and Agarwala, 1975), confirming the thioenol structure of metal-MBT compounds.

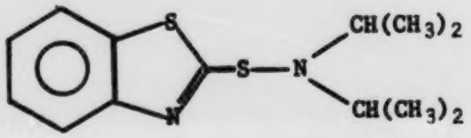
The products of reactions of MBT involving the replacement of the mercapto hydrogen have the general structure:



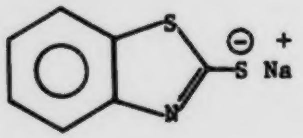
When R contains sulfur as the bonding atom, the compound is a disulfide such as 2,2'-dithiobisbenzothiazole (MBTS):



When an S-N bond is present, the compound is a sulfenamide, such as N,N-diisopropyl-2-benzothiazolesulfenamide:



The mercapto proton of MBT can be readily replaced by metal ions, yielding salts, typical of which is sodium mercaptobenzothiazole (NaMBT):



This review emphasizes MBT and those derivatives of MBT that appear to have commercial or environmental significance. Table 1 contains a list of the names of the commercial mercaptobenzothiazole compounds, their chemical structures, and acronyms which have been used throughout this report to refer to these materials. All of the compounds are produced in commercial quantities, according to the available chemical marketing literature (SRI, 1975; last two years - 1972, 1973 of the U.S. International Trade Commission [USITC, 1959-73]).

2. Physical Properties of the Pure Material

2-Mercaptobenzothiazole and its derivatives are remarkably similar in their physical properties, with the exception of subtle color differences and melting points, as is inducated in Table 2. A relatively wide softening-melting range is common, probably indicative of purity variations in the commercial products. The compounds are all powders, light in color, with characteristic odors, generally soluble in polar solvents but insoluble

Table 1. Commercially Important Mercaptobenzothiazole Compounds

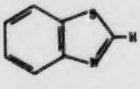
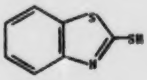
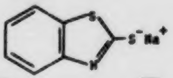
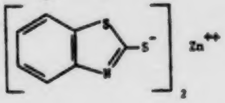
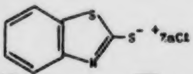
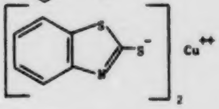
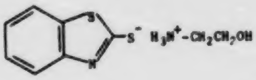
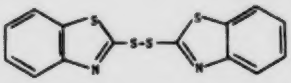
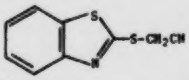
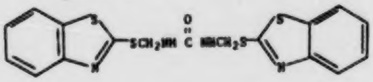
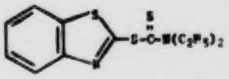
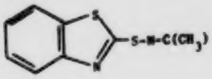
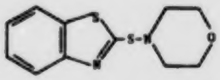
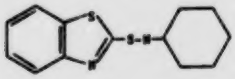
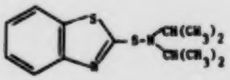
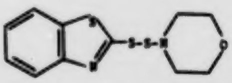
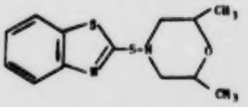
Chemical Name	Structure	Acronym
Benzothiazole		
2-Mercaptobenzothiazole		MBT
2-Mercaptobenzothiazole, sodium salt		NaMBT
2-Mercaptobenzothiazole, zinc salt		ZMBT
2-Mercaptobenzothiazole, zinc chloride		
2-Mercaptobenzothiazole, copper salt		
2-Mercaptobenzothiazole, monoethanolamine salt		
2,2'-Dithiobisbenzothiazole		MBTS
Cyanomethylthiobenzothiazole		
1,3-Bis(2-benzothiazolylmercaptomethyl)urea		
2-Benzothiazyl-N,N-diethylthiocarbamyl sulfide		
N-*tert*-Butyl-2-benzothiazolesulfenamide		TBBS
N-Oxydiethylene-2-benzothiazolesulfenamide		OBS
N-Cyclohexyl-2-benzothiazolesulfenamide		CBS
N,N-Diisopropyl-2-benzothiazolesulfenamide		DIBS
4-Morpholinyl-2-benzothiazyl disulfide		
N-(2,6-Dimethylmorpholino)-2-benzothiazolesulfenamide		

Table 2. Physical Properties of 2-Mercaptobenzothiazoles and Selected Derivatives [a]

	Molecular Weight	Physical State	Color	Odor	Specific Gravity ±0.03	Melting Range °C	Water	Nonaqueous		Sources
								Polar	Nonpolar	
2-Mercaptobenzothiazole	167	powder	light yellow-tan	distinct, characteristic	1.50	177-178	0.25g/100g[b]	S	SS	1,2,3,4
2,2'-Dithiobisbenzothiazole	333	powder	cream to light yellow	slight	1.54	160-180	I	SS	SS	1,2,4
N-*tert*-Butyl-2-benzothiazolesulfenamide	238	powder	light tan	characteristic	1.29	104-112	I	S	S	1,3
N-Oxydiethylene-2-benzothiazolesulfenamide	252	flakes	tan		1.37	75- 90	I	S	S	1,2,3
N-Cyclohexyl-2-benzothiazolesulfenamide	264	flakes	greenish-tan		1.29	94-108	I	SS	SS	1,4
N,N-Diisopropyl-2-benzothiazolesulfenamide	266	flakes	greenish-yellow	characteristic	1.21	53- 60	I	S	S	1
Sodium Mercaptobenzothiazole	189	solid					S			1,2,3
Zinc Mercaptobenzothiazole	398	powder	cream		1.72	>300	I	I	SS	1,2,4

---

[a] The information in this table was obtained from product bulletins supplied by:

1 American Cyanamid Company, Bound Brook, New Jersey (1970)

2 R.T. Vanderbilt Company, Norwalk, Connecticut (1974)

3 Uniroyal Chemical, Naugatuck, Connecticut (1972)

4 B.F. Goodrich Chemical Company, Cleveland, Ohio (1975)

[b] Davis, 1980

in water (except for the sodium salt). All are stable materials, without unusual storage or handling hazards.

Figure 1 shows the ultraviolet absorption spectrum of MBT in acidic, neutral, and basic aqueous solution. Figure 2 shows the UV spectrum of MBT in chloroform. The λ_{max} shifts can be attributed to the different perturbations resulting from the dissociated and undissociated forms in acidic, basic, or neutral conditions. The characteristic peak at 329 nm in chloroform is utilized in recently reported assay techniques for MBT (Jones and Woodcock, 1975).

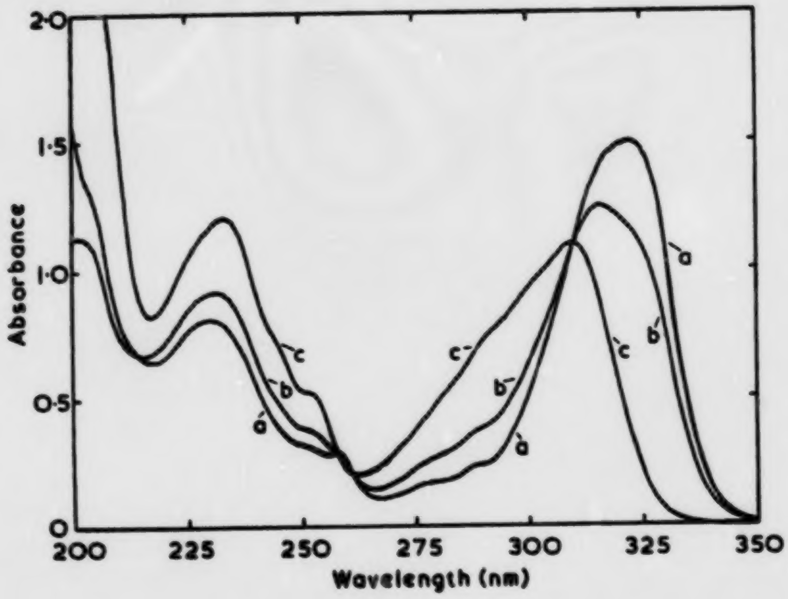


Figure 1. UV Absorbance Curves For Aqueous Solution of 10 ppm MBT. a. pH 1.6, b. pH 6.8, c. pH 11.7

(Jones and Woodcock, 1973)

(Reprinted with permission from the Department of Energy, Mines & Resources)

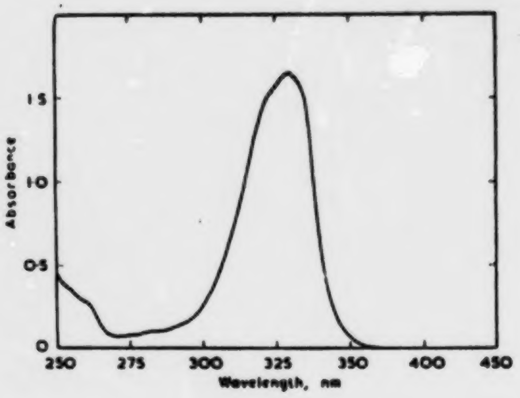


Figure 2. UV Absorbance Spectrum of 10 mg/l. MBT in Chloroform Using 1-cm Cells (Jones and Woodcock, 1975) (Reprinted with permission from the American Chemical Society)

3. Properties of the Commercial Materials

MBT commercial materials have the same physical properties listed in Table 2. In addition, the specifications listed in Table 3 apply to commercial materials as supplied by the manufacturers.

Table 3. Commercial Specifications for 2-Mercaptobenzothiazole and Selected Derivatives

	Moisture, % max.	Ash, % max.	Assay, % min.	Decusting Agent, % max.	Fineness (Wet Method) (100 mesh) % min.	Pet. Ether Extract, %	Manufacturer or Distributor	Commercial Name	Free MBT, % max.	Free MBTS, % max.
2-Mercaptobenzothiazole	0.5	0.5	92	2.5	99.9		Amer. Cyanamid	MBT		
	1.0	0.5			99.8	1-3	Vanderbilt	Captax		
	1.0	0.5			99.8		Goodrich	MBT		
2,2'-Dithiobisbenzothiazole	0.5	1.0	91	2.5	99.9		Amer. Cyanamid	MBTS	2	
	0.7	1.0			99.9	1-3	Vanderbilt	Altax		
	1.0	0.7			99.9		Goodrich	MBTS	5	
N-*tert*-Butyl-2-benzothiazolesulfenamide	0.5	0.5			100		Amer. Cyanamid	TBBS		1
N-Oxydiethylene-2-benzothiazolesulfenamide	0.2	0.5			100			NOBS		3
	0.5	0.5			100		Vanderbilt	Amax		3
	0.5	0.15					Goodrich	OBTS		
Zinc 2-Mercaptobenzothiazole	1.0		15% Zn		99.9		Amer. Cyanamid	ZMBT		
	1.0		15-18% Zn		99.9		Vanderbilt	Zetax		
	2.0		15-18% Zn		99.8		Goodrich	ZMBT		

Sodium 2-mercaptobenzothiazole is supplied as a 50% aqueous solution, unlike other MBT chemicals which are insoluble in water and are supplied as solids. Specifications for the NaMBT solutions of three manufacturers are listed in Table 4.

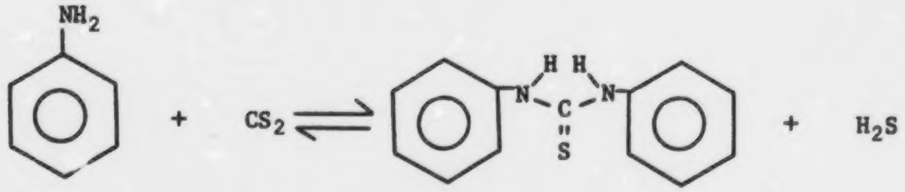
Table 4. Commercial Specifications for Sodium MBT Solution, 50% Aqueous

Manufacturer	Color (Garner) max.	Clarity	Specific Gravity	Assay %	NaOH %	Cl^-, % max.	SO_4^{-2}, % max.
American Cyanamid	10	clear	1.25-1.29 (15.5°C)	50.0 ± 0.5	0.1-0.5	0.1	0.[illegible]
Uniroyal	10		1.27 (15°C)	50.0	0.3	nil	0.15
R.T. Vanderbilt		clear, amber	1.255 (25°C)	50	0.5		

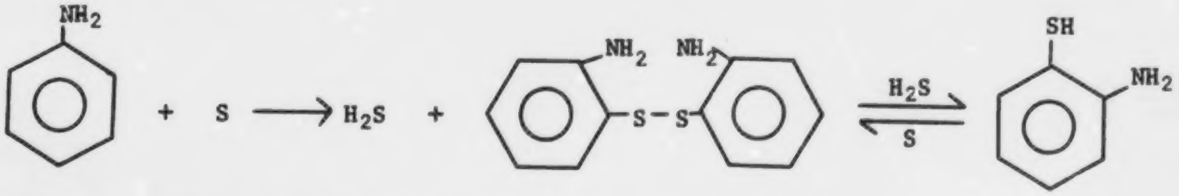
4. Principal Contaminants of Commercial Products

The major starting material from which MBT and its derivatives are manufactured is aniline (Kouris and Northcott, 1963). Aniline heated with equimolar amounts of sulfur and carbon disulfide at temperatures of 200-275°C (Allen, 1966) yields MBT and possibly other products whose contamination of the major product is dependent on the temperature range of the reaction medium. Besides by-products, unreacted starting materials, if any, may contaminate the desired product.

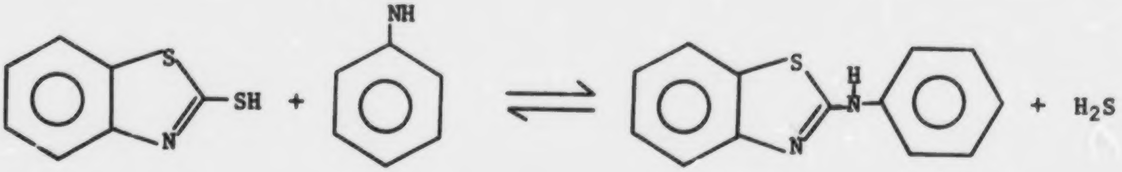
Ivanova and Shebuev (1957) have analyzed the main and side reactions which lead to the formation of MBT. The first reaction is observed when a mixture of aniline, CS_2, and S is heated to 170°C. Aniline reacts with CS_2 to form thiocarbanilide:



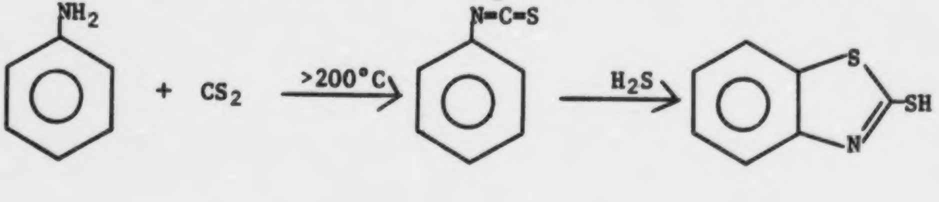
This reaction is reversible as long as H_2S is present and, in fact, tends to shift to the left because thiocarbanilide is unstable above 160°C. Above 170°C, the removal of thiocarbanilide is further favored as aniline reacts irreversibly with sulfur to form 2,2'-diaminodiphenyl disulfide, which in the presence of H_2S forms 2-aminothiophenol:



Both the disulfide and 2-aminothiophenol react readily with CS_2 to form MBT. At temperatures above 220°C, MBT reacts with aniline to form anilinobenzothiazole:



Above 260°C MBT is unstable and decomposes to form benzothiazole. Above 200°C, aniline and CS_2 react to form, in addition to thiocarbanilide, phenylisothiocyanate, which is very unstable in the presence of H_2S, and reacts to form MBT:



Since most of the side reactions lead to by-products which are unstable under the conditions in which they are formed and decompose to form the original starting materials or MBT, it is not surprising that the by-products are notably absent from the final product when the reaction temperatures are carefully controlled. If the reaction medium were allowed to rise above 260°C, benzothiazole would be an expected contaminant of the desired product. If the reaction is quenched by rapidly lowering the temperature, the by-products shown in the side reactions can be identified (Ivanova and Shebuev, 1957).

MBT and its derivatives are considered to have excellent storage and handling stability; contamination due to decomposition during storage is not likely (American Cyanamid Co., 1970).

There remains then the possibility of contamination due to impurities in the starting materials as well as the presence of unreacted starting materials. American Cyanamid Co. (1970) is the only manufacturer of these materials which publishes minimum purity specifications (for MBT and MBTS only, 92% and 91% minimum assay, respectively [other manufacturers have indicated that the assay is usually between 95-97% purity]). The minimum purity specifications indicate that if approximately 5% of the contents is assumed to be ash, moisture, and dedusting agent, approximately 3% of the product contents remain unaccounted for. This 3% may consist of unreacted starting materials and by-products. MBT may be a major contaminant of the derivatives (up to 5%, see Table 3). American Cyanamid Co. (1970) also specifies the possible presence of small quantities of Cu (< 10 ppm) and Mn (< 10 ppm) in the compounds listed in Table 3, as well as Fe (< 0.1%) in all except ZMBT. It is probable that the products of other manufacturers have comparable purity and contamination levels since the other published specifications are essentially identical.

B. Chemistry

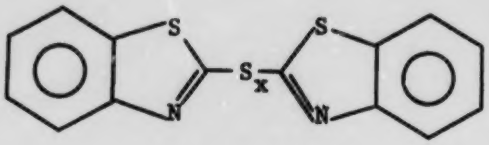
1. Reactions Involved in Uses

2-Mercaptobenzothiazole finds major uses in three distinct and quite different areas. It is used in the rubber industry as a vulcanization accelerator (Nathan, 1965) and antioxidant; in metal processing and applications (especially in the automobile industry) as an anticorrosion agent (Turk, 1969); and as a fungicide and bacteriostatic agent (Turner, 1966). In addition, there are a number of minor uses for MBT and its derivatives and related compounds, as a chemical intermediate and in analytical applications as a metal chelating agent (Shimidzu and Uno, 1973). Its chemistry as an intermediate has been of theoretical and practical interest because many natural products and drugs contain the thiazole ring (e.g., penicillin, sulfathiazole, sulfonamides, thiamine) (Allen, 1966).

Vulcanization is a crosslinking process in which sulfur bridges are formed between the unsaturated portions of polymers which make up an unvulcanized rubber (Winspear, 1958). In order for vulcanization to proceed readily, elemental sulfur, which exists at ambient temperatures in the ring form, S_8, must be converted into a biradical chain. This can be accomplished by heating rubber and sulfur at high temperatures for long periods of time, but such treatment often leads to unpredictable and undesirable properties in the vulcanized rubber. It is the function of an accelerator such as MBT to reduce both the temperature and time requirements of vulcanization, thus aiding in the production of a product with uniform and predictable properties.

The ideal accelerator allows vulcanization to occur reproducibly and rapidly at a given temperature, while restraining vulcanization during preparative operations (i.e., milling, compounding, etc.) (Shaver, 1968).

MBT and its derivatives all function in similar fashion as accelerators. The main difference between these compounds is the temperature at which vulcanization begins, and the delay time before vulcanization at a given temperature. The mechanism of vulcanization involves the decomposition and recombination of these compounds at high temperatures (Dogadkin et al., 1961). In the presence of elemental sulfur, S_8, polysulfides are also formed, an example of which for MBT accelerator systems is (Coran, 1965):



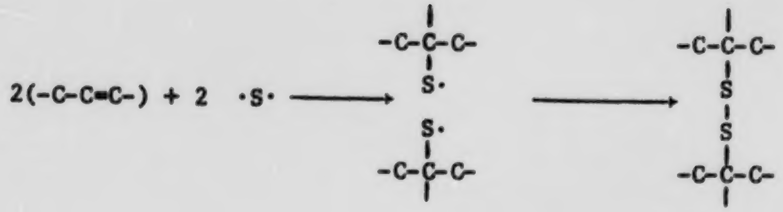
These polysulfides split and recombine to form atomic sulfur and other free radicals (Winspear, 1958):

$$R\text{-}S\text{-}S\text{-}R \longrightarrow 2R\text{-}S\cdot \longrightarrow R\text{-}S\text{-}R + \cdot S\cdot$$

The free radicals attack S_8 rings forming sulfur chains (Winspear, 1958):

$$R\text{-}S\cdot + S_8 \longrightarrow R\text{-}S_7\text{-}S\cdot$$

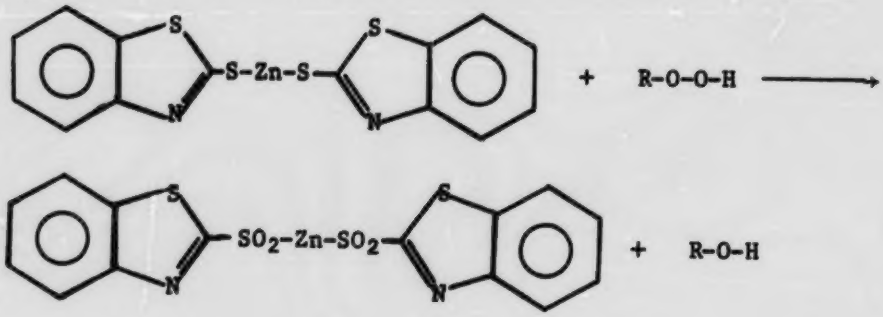
The chains eventually fragment into atomic sulfur which reacts immediately with the rubber polymer, forming the crosslinks characteristic of vulcanized rubber (Winspear, 1958):



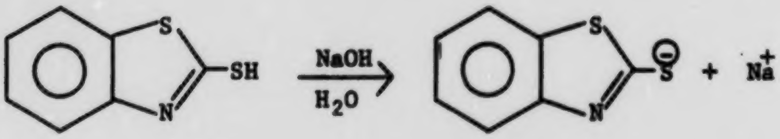
$$2(\text{-C-C=C-}) + 2\ \cdot\text{S}\cdot \longrightarrow \begin{array}{c} | \\ \text{-C-C-C-} \\ | \\ \text{S}\cdot \\ \\ \text{S}\cdot \\ | \\ \text{-C-C-C-} \\ | \end{array} \longrightarrow \begin{array}{c} | \\ \text{-C-C-C-} \\ | \\ \text{S} \\ | \\ \text{S} \\ | \\ \text{-C-C-C-} \\ | \end{array}$$

A complex mixture of MBT compounds and MBT by-products results from the vulcanization step. However, no quantitative study of MBT products formed during vulcanization has been undertaken. MBT will thermally decompose to benzothiazole at temperatures greater than 260°C (Ivanova and Shebuev, 1957), and this compound has been detected in volatiles from simulated vulcanization processes (Rappaport, 1975) and in rubber processing plant effluents (Webb *et al.*, 1975). Rappaport (1975) also detected *t*-butylisothiocyanate which he suggested was a breakdown product of *t*-butyl-2-benzothiazolesulfenamide.

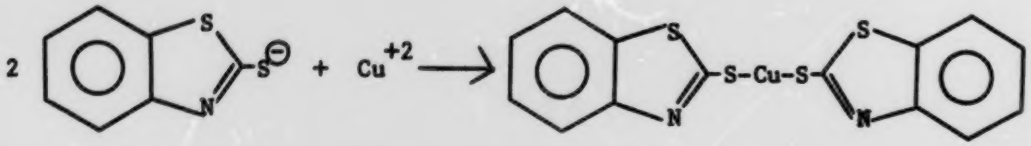
Zinc mercaptobenothiazole (ZMBT) is an excellent antioxidant when used in rubber formulations. If not actually introduced during compounding, ZMBT will form during vulcanization if MBT (or a potential precursor such as 2,2'-dithiobisbenzothiazole) and zinc oxide are in the formulation (the latter usually is). The basis of its antioxidant activity is the ability of ZMBT to react readily with those hydroperoxides which normally form in the rubber substrate during aging and which are the cause of degradation of the rubber (Brooks, 1963). In the course of the reaction of ZMBT with hydroperoxides, the salt is oxidized to the sulfonate, while the hydroperoxides are reduced to alcohols:



Although insoluble in acidic or neutral aqueous media, MBT is a weak acid in water and dissolves in basic solutions:

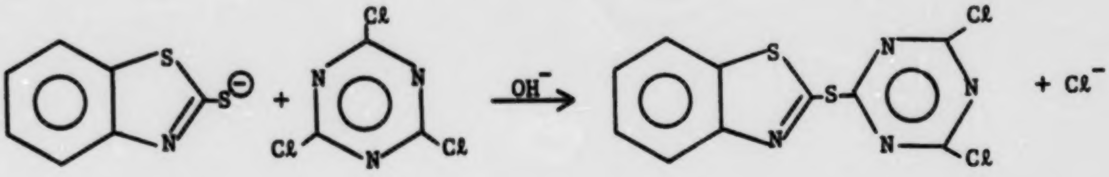


The anion complexes readily with a wide range of metal ions, including Al^{+3}, Cu^{+2}, Fe^{+3}, and Zn^{+2}, to produce insoluble complex salts which, when formed as coatings on a metal surface, protect the surface against further oxidation (Prajapati et al., 1972):



This is believed to be the chemical basis for the activity of MBT as an anticorrosion agent and may be the basis of its toxicity when administered intravenously. By complexing readily with Cu^{+2}, MBT inhibits the action of the enzyme dopamine β-hydroxylase which catalyzes the conversion of dopamine to noradrenaline, a neural transmitter (Johnson et al., 1970) (see Section III-B-2, p. 83 for more details).

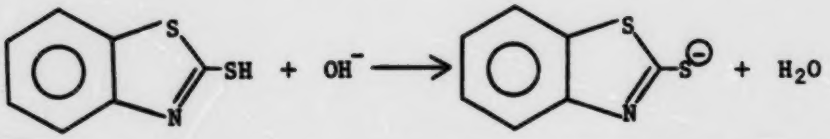
It is thought that, in order for MBT to act as a fungicide, the molecule must penetrate the cell membranes of the fungus and act within the cells (Bowes et al., 1970). In order to do this, the fungitoxic molecule must not be bound to the substrate (textile, leather, etc.) which it is protecting. At the same time, it must not have such freedom of migration that it is easily leached out of the substrate, affording a protection time that is unreasonably short. Bowes and his colleagues (1970) have shown that MBT can be bound to the collagen in leather using cyanuric chloride as an intermediary. MBT reacts with the cyanuric chloride under basic conditions to form 1-(2-mercaptobenzothiazolyl)-3,5-dichlorotriazine:



The chlorine atoms of the MBT derivative can react with the amino groups in lysine residues in leather, thus binding the compound to the leather. Over a period of time the derivative eventually breaks down to form MBT which protects the leather from fungal attack (Bowes et al., 1970). The production of free MBT increases in hot or moist conditions, exactly the circumstances which most favor fungal growth.

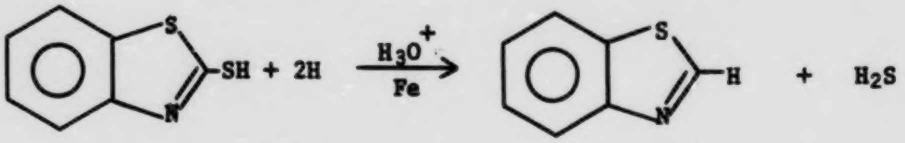
2. Hydrolysis, Oxidation and Photochemistry

MBT is insoluble in aqueous solutions at or below pH 7. In basic solution, MBT hydrolyzes according to the equation:



Many metallic salts of MBT can be prepared by simply adding the metal ion to a basic solution of MBT. Most metal salts of MBT are insoluble in water and immediately precipitate out, the sodium salt being a major exception.

Because MBT is used as a corrosion inhibitor, its stability in aqueous media is quite important. Weibull (1962) has found that the stability of MBT is affected by the presence of iron. While stable in neutral or basic solution in the presence of iron, in acidic media MBT is reduced in the presence of iron to benzothiazole:



MBT and its derivatives are quite resistant to oxidation in air, and NaMBT in aqueous solution is not oxidized even at temperatures of 100°C (Squires, 1958). Under fire conditions, the principal products of combustion are carbon dioxide, sulfur dioxide, and water (Shaffer, 1971a,b,c,d). Incomplete combustion will produce carbon monoxide, and the combustion of sulfenamides tends to produce nitrogen oxides as well.

The mercapto group is subject to oxidation by hydroperoxides, yielding sulfonates (see Section I-B-1, p. 14), as are the sulfur bridges in MBTS and other polysulfide derivatives.

In the presence of ozone and potassium iodide, MBT dimerizes to MBTS (Petrenko et al., 1975). This reaction is favored by lowering the temperature to about 0°C. The dimer can then be further oxidized to the disulfonate if sufficient ozone is available.

MBT absorbs ultraviolet light strongly at about 328 nm (see Figures 1, 2), and, therefore, will strongly absorb sunlight (> 290 nm). However, no evidence is available to indicate whether the energy absorbed can be efficiently translated into photochemical processes. The fact that manufacturers have not noted any light sensitivity for mercaptobenzothiazole compounds would suggest that these compounds are not photochemically reactive, at least under conditions of storage, transport, or use (usually in a solid form).

II. Environmental Exposure Factors

A. Production, Consumption

1. Quantity Produced, Exported and Imported

More than 100 million pounds of mercaptobenzothiazole compounds valued at over $60 million are produced annually (see Table 5) and are mainly consumed in rubber vulcanization operations. The parent compound, 2-mercaptobenzothiazole, accounts for less than 10% of the total production. The largest volume compound is 2,2'-dithiobisbenzothiazole (23.2 million pounds in 1973), followed by the sodium salt of MBT (11.9 million pounds in 1973). According to industry sources (personal communication), approximately one-half of the NaMBT produced is used in anticorrosion applications and, therefore, is not included in the totals of rubber-processing thiazoles. The other half is used mostly in synthesizing other MBT derivatives. Over the years, the production quantities of N-cyclohexyl-2-benzothiazolesulfenamide and the zinc salt of MBT have also been enumerated (see Table 5). In 1972, the production of "other rubber-processing thiazoles" amounted to 46 million pounds. This category consisted of nine MBT derivatives (see Table 5 for list) and one non-MBT derivative (thiazoline-2-thiol). However, in 1973, one MBT derivative [1,3-bis(2-benzothiazolylmercaptomethyl)urea] and thiazoline-2-thiol were no longer included in the category (USITC, 1973) and, therefore, their production volumes are probably quite small. Thus, the 46 million pounds noted in 1972 for the "other rubber-processing" category is mostly attributable to eight MBT derivatives. Figure 3 graphically illustrates total rubber thiazole accelerator production trends in the United States compared to total accelerator production.

In 1971, approximately 11.6 million pounds of cyclic rubber accelerators were exported (SRI, 1972a). Since, in that year, thiazole derivatives

Table 5. Production and Sales of 2-Mercaptobenzothiazole Compounds (U.S.I.T.C., 1959–1973. 1974p, SRI, 1972a)

Millions of Pounds

	Sodium 2-Mercapto-benzothiazole		N-Cyclohexyl-2-benzothiazole-sulfenamide		2-Mercapto-benzothiazole		2,2'-Dithiobis-benzothiazole		Zinc 2-Mercapto-benzothiazole		Other rubber-processing thiazoles[a,b]		Total, all rubber-processing thiazoles[a]	
Year	Pro-duction	Sales	Pro-duction	Sales	Pro-duction	Sales	Pro-duction	Sales	Pro-duction	Sales	Pro-duction	Sales	Pro-duction	Sales
1940	– –	– –	– –	– –	5.4	– –	– –	– –	– –	– –	– –	– –	– –	– –
1941	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
1942	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
1943	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
1944	– –	– –	– –	– –	11.2	6.0	– –	– –	– –	– –	17.6	– –	28.8	23.1
1945	– –	– –	– –	– –	14.8	7.9	– –	– –	– –	– –	19.5	– –	34.3	26.3
1946	– –	– –	– –	– –	16.5	8.6	8.3	8.8	– –	– –	12.5	12.7	37.3	30.1
1947	– –	– –	– –	– –	17.7	9.6	12.4	10.8	– –	– –	11.1	10.9	41.2	31.4
1948	– –	– –	– –	– –	15.3	2.6	9.8	7.5	– –	– –	10.5	17.0	35.6	19.6
1949	– –	– –	– –	– –	11.5	1.9	10.3	7.9	– –	– –	9.0	7.9	30.8	17.7
1950	– –	– –	– –	– –	14.9	2.9	15.0	11.5	– –	– –	10.0	10.0	39.8	24.4
1951	– –	– –	– –	– –	18.2	3.2	17.1	12.7	– –	– –	11.9	11.2	47.2	27.2
1952	– –	– –	– –	– –	14.9	2.2	15.0	9.5	– –	– –	– –	– –	– –	– –
1953	– –	– –	– –	– –	15.9	2.7	14.9	10.1	– –	– –	22.0	12.2	52.8	24.0
1954	– –	– –	– –	– –	11.9	3.9	14.7	10.6	– –	– –	20.4	10.3	47.0	24.8
1955	– –	– –	– –	– –	18.5	5.4	15.4	10.4	– –	– –	21.4	21.3	55.3	37.0
1956	– –	– –	– –	– –	15.7	3.2	18.2	11.0	– –	– –	19.2	17.6	53.1	31.9
1957	– –	– –	– –	– –	– –	– –	16.1	10.0	– –	– –	43.4	21.1	59.5	31.1
1958	– –	– –	– –	– –	13.3	2.6	15.2	9.1	– –	– –	26.8	16.3	55.4	28.0
1959	– –	– –	6.4	5.7	6.9	4.1	19.5	11.6	– –	– –	26.0	14.2	58.8	35.7
1960	– –	– –	5.7	5.2	7.2	4.4	17.5	10.1	– –	– –	25.2	14.0	55.6	33.7
1961	– –	– –	7.4	5.8	6.4	4.1	16.7	9.8	– –	– –	24.8	14.8	55.3	34.5
1962	– –	– –	7.8	6.4	7.0	4.6	18.1	10.0	– –	– –	27.6	16.2	60.5	37.2
1963	– –	– –	7.2	5.8	7.1	5.7	18.4	9.7	– –	– –	29.8	18.6	62.5	39.8
1964	– –	– –	10.1	5.7	8.0	5.0	21.0	10.3	– –	– –	35.9	21.2	75.0	42.2
1965	– –	– –	7.2	6.2	– –	– –	21.3	10.8	– –	– –	30.9	27.0	59.4[a]	44.1
1966	25.6[c]	– –	6.6	4.4	6.3	– –	23.3	10.9	– –	– –	28.9	29.8	65.1	45.1
1967	– –	– –	4.7	3.4	4.7	– –	21.9	10.6	– –	– –	25.4	29.4	56.7	43.4
1968	– –	– –	4.9	3.7	6.1	– –	22.8	11.6	4.4	– –	31.9	37.5	70.1	52.8
1969	– –	– –	4.9	3.4	6.7	4.8	23.5	11.6	4.6	3.6	36.5	32.8	76.0	56.3
1970	– –	– –	5.9	4.7	6.9	5.2	23.2	11.0	4.8	4.1	36.4	33.3	77.3	58.4
1971	– –	– –	7.6	4.6	6.8	4.2	21.1	10.5	– –	– –	45.1	39.9	80.6	59.2
1972	9.7	– –	8.8	5.2	6.0	4.7	21.3	11.7	4.0	4.1	46.2	41.3	86.3	67.0
1973	11.9	5.8	– –	6.6	7.9	7.6	23.2	12.9	– –	4.5	63.5	42.9	94.6	74.5
1974	– –	– –	4.6	3.4	6.1	5.3	20.7	11.5	– –	– –	52.1	45.0	83.5	65.3

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[a] After 1964, sodium 2-mercaptobenzothiazole was not classified as a rubber-processing chemical.

[b] Compounds in the category of other rubber processing thiazoles vary slightly over the years. In 1972, the category included 2-benzothiazyl-N,N-diethylthiocarbamoyl sulfide; 1,3-bis(2-benzothiazolylmercaptomethyl)urea; N-*tert*-butyl-2-benzothiazolesulfenamide; N,N-diisopropyl-2-benzothiazolesulfenamide; N-(2,6-dimethylmorpholino)-2-benzothiazolesulfenamide; 2-mercaptobenzothiazole, copper salt; 4-morpholinyl-2-benzothiazyl disulfide; N-oxydiethylene-2-benzothiazolesulfenamide; and thiazoline-2-thiol. Only the last compound is not a MBT derivative. When production or sales volumes are not listed under individual compounds, they are included in the other category.

[c] SRI (1972)

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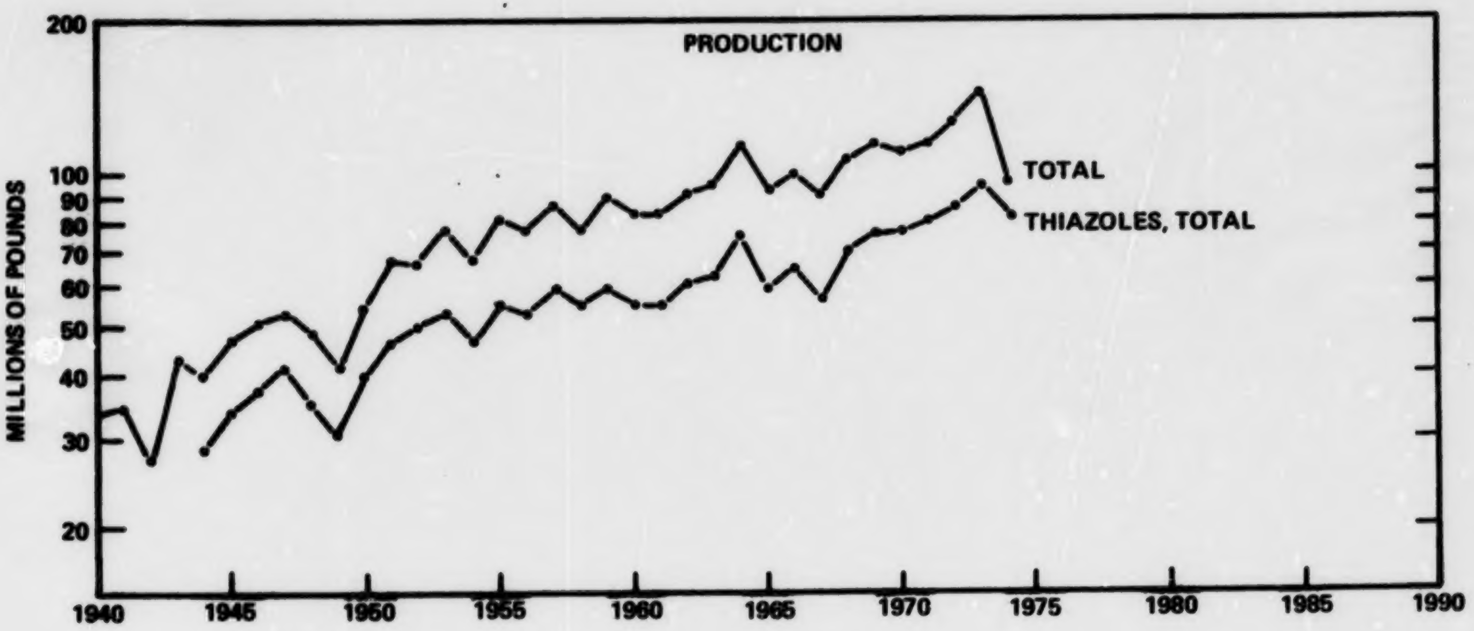


Figure 3. Organic Rubber Accelerators Production and Sales (SRI, 1972a)

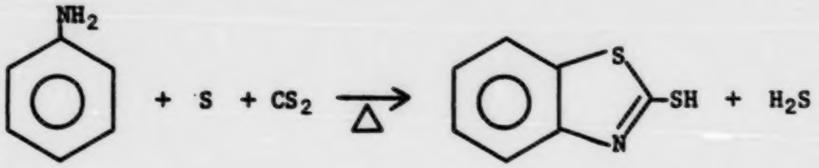
totalled 81 million pounds compared to a 93 million pound total for cyclic accelerators, it appears that substantial quantities of MBT derivatives were probably exported. Imports of MBT compounds have been relatively small. For example, in 1973 only 22,046 and 73,413 pounds of MBTS and MBT, respectively, were imported (U.S. Tariff Commission, 1974).

2. Producers and Production Sites

The major manufacturers of MBT compounds of any economic significance are listed in Table 6. They are located mostly in the eastern part of the country, close to their major market, tire manufacturers. A significant quantity of these chemicals is manufactured by the tire producers themselves for their own internal consumption and never reaches the open market.

3. Production Methods and Processes

2-Mercaptobenzothiazole is manufactured by reacting aniline with a solution of sulfur in carbon disulfide (for the chemistry of this reaction, see Section I-A-4, p. 9):



This can be accomplished in a continuous process using the system invented by Cooper and Mensing (1951) as illustrated in Figure 4. Column A represents a high pressure reactor into which aniline and a solution of sulfur in carbon disulfide are simultaneously fed, both liquids having been preheated to about 240°C. The pressure in the column is kept at about 1,000 psi. Either a curved deflection plate or an impeller (as shown at C) assures a uniform upward flow

Table 6. Manufacturers of Mercaptobenzothiazole (SRI, 1975)

Compound	Company	Location	Trade Name
2-Mercaptobenzothiazole	American Cyanamid	Bound Brook, New Jersey	MBT
	DuPont	Deepwater Point, New Jersey	MBT
	Goodrich	Henry, Illinois	Goodrite MBT
	Goodyear	Niagara Falls, New York	MBT
	Monsanto	Nitro, West Virginia	Metrax
	Pennwalt	Wyandotte, Michigan	MBT
	Uniroyal	Geismar, Louisiana	MBT-UO
2,2'-Dithiobisbenzothiazole	American Cyanamid	Bound Brook, New Jersey	MBTS
	DuPont	Deepwater Point, New Jersey	MBTS
	Goodrich	Henry, Illinois	Goodrite MBTS
	Goodyear	Akron, Ohio	MBTS
		Niagara Falls, New York	
	Monsanto	Nitro, West Virginia	Thiofide
	Pennwalt	Wyandotte, Michigan	MBTS
	Uniroyal	Geismar, Louisiana	MBTS
2-Mercaptobenzothiazole, sodium salt	American Cyanamid	Bound Brook, New Jersey	Sodium MBT solution
	Buckman Labs	Cadet, Missouri	
		Memphis, Tennessee	
	Goodyear	Akron, Ohio	
	Monsanto	Nitro, West Virginia	
	Uniroyal	Geismar, Louisiana	Sodium MBT
2-Mercaptobenzothiazole, copper salt	American Cyanamid	Woodbright, New Jersey	
2-Mercaptobenzothiazole, zinc salt	American Cyanamid	Bound Brook, New Jersey	
	DuPont	Deepwater Point, New Jersey	
	Goodrich	Henry, Illinois	Goodrite ZMBT
	Goodyear	Niagara Falls, New York	
	Uniroyal	Geismar, Louisiana	OXAF
	Vanderbilt	Bethel, Connecticut	Zetrax

Table 6. (cont'd)

Compound	Company	Location	Trade Name
2-Mercaptobenzothiazole, zinc chloride	DuPont	Deepwater Point, New Jersey	
2-Mercaptobenzothiazole, monoethanolamine salt	Vanderbilt	Bethel, Connecticut	
Cyanomethylthiobenzothiazole	Millmaster Oryx	Jersey City, New Jersey	
1,3-Bis(2-benzothiazolylmercaptomethyl) urea	Lakeway Chemicals	Muskegon, Michigan	
	Monsanto	Nitro, West Virginia	
2-Benzothiazyl-N,N-diethylthiocarbamyl sulfide	Pennwalt	Wyandotte, Michigan	
N-*tert*-Butyl-2-benzothiozolesulfenamide	Monsanto	Nitro, West Virginia	
	Pennwalt	Wyandotte, Michigan	
	Uniroyal	Geismar, Louisiana	Delac NS
N-Oxydiethylene-2-benzothiazolesulfenamide	American Cyanamid	Bound Brook, New Jersey	Goodrite OBTS
	Goodrich	Henry, Illinois	
	Goodyear	Akron, Ohio	
	Pennwalt	Wyandotte, Michigan	
N-Cyclohexyl-2-benzothiazolesulfenamide	American Cyanamid	Bound Brook, New Jersey	Cydac
	DuPont	Deepwater Point, New Jersey	Conac S
	Goodrich	Henry, Illinois	Goodrite CBTS
	Monsanto	Nitro, West Virginia	Santocure
	Pennwalt	Wyandotte, Michigan	
	Uniroyal	Geismar, Louisiana	Delac S
N,N-Diisopropyl-2-benzothiazolesulfenamide	American Cyanamid	Bound Brook, New Jersey	
4-Morpholinyl-2-benzothiazyl disulfide	Goodyear	Akron, Ohio	
N-(2,6-Dimethylmorpholino)-2-benzothiazolesulfenamide	Monsanto	Nitro, West Virginia	

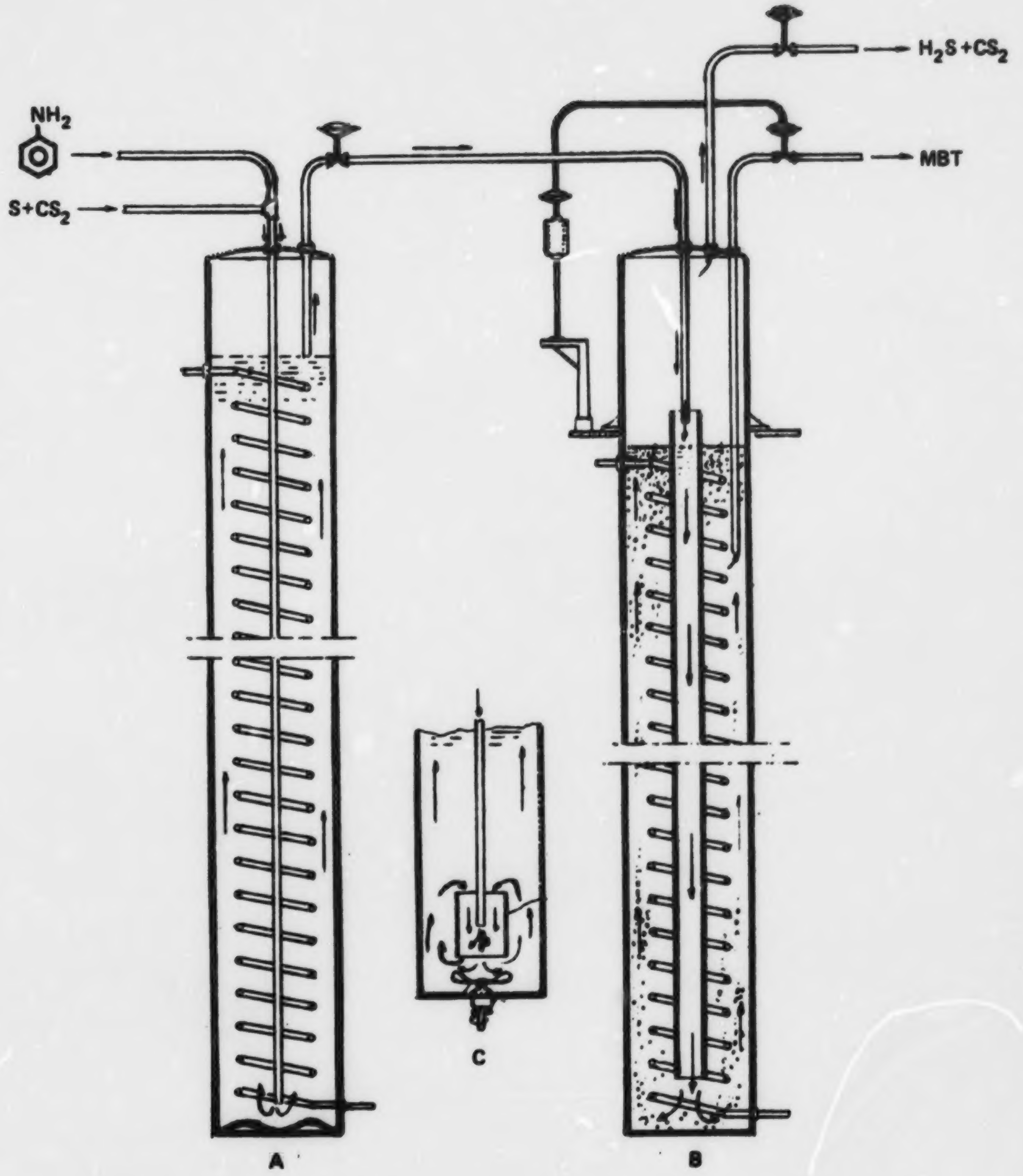

NH$_2$
S+CS$_2$
H$_2$S+CS$_2$
MBT
A
B
C

Figure 4. Manufacture of Mercaptobenzothiazole (Cooper and Mensing, 1951)

of the reactants and products. The evolution of hydrogen sulfide gas also maintains agitation of the reaction medium. As reactants are continually added to Column A, the products are discharged continually into separator Column B. The material in Column B consists of molten MBT, H_2S, and CS_2 (an excess of CS_2 is used in Column A). The temperature of both columns is kept at about 260°C with high pressure steam coils which can be seen in the figure. The pressure in Column B is kept at about 350 psi, about a third that of Column A. The lower pressure and high temperature in Column B assure that both the H_2S and CS_2 will be in the gaseous state. A weight-responsive apparatus on Column B discharges the gases to maintain the proper pressure. After leaving Column B, the gases are cooled to 20-40°C at constant pressure and then allowed to expand adiabatically to atmospheric pressure, reducing the temperature to about -40°C. This liquefies only the CS_2, which is thereby separated from the H_2S and recirculated. Meanwhile, liquid MBT is continually tapped from Column B at about atmospheric pressure and cooled to the solid state.

Once MBT has been manufactured, the sodium salt is easily obtained by dissolving the MBT in an aqueous solution of NaOH. Insoluble metal mercaptobenzothiazoles (zinc, copper, etc.) are formed by the addition of the appropriate cation to aqueous solutions of NaMBT, whereupon the metal-MBT compound precipitates out of the solution.

MBT is also a starting material for producing a number of disulfides, such as MBTS, when manufactured according to the method of Kleiman (1950). In this method, an alkali metal mercaptide is used as a catalyst for the reaction between MBT and disulfides (e.g., methyl disulfide); the product consists of MBTS and the volatile mercaptan. Other symmetrical as well as unsymmetrical disulfides can also be synthesized by this method, depending upon the choice of disulfide(s) (and thiol) used as starting materials.

MBTS in turn is the starting material for the manufacture of N,N-diispropyl-2-benzothiazolesulfenamide. MBTS is reacted with diisopropyl amine and diisopropylchloroamine on a steam bath for 30 minutes, after which the product crystallizes out when the reaction medium is cooled on an ice bath. It is washed with cold water and dried to give a high purity product (99%) (Hardman, 1956).

N-Oxydiethylene-2-benzothiazolesulfenamide is prepared by reacting MBT and morpholine in benzene or toluene in the presence of a chlorinating agent such as sodium hypochlorite. The reaction medium is then treated with 50% aqueous NaOH, and the product is removed from the organic layer by evaporation (Sullivan, 1956).

N-Cyclohexyl-2-benzothiazolesulfenamide may be prepared by oxidizing cyclohexylamine and MBT with NaOCl at 45-70°C. The product forms as a solid which is washed with water and dried (Lunt, 1956).

More recently, a number of sulfenamides have been manufactured by oxidation of MBT using metal phthalocyanines as catalysts with the appropriate amine (Campbell and Wise, 1973).

4. Market Price

Figure 5 illustrates the past and present prices of MBT and MBT derivatives. The prices have stayed fairly constant over the years plotted, with the exception of N-cyclohexyl-2-benzothiazolesulfenamide. The reason for the sharp rise in price for that compound is unknown. The cheapest product is MBT ($0.36/lb in 1973), probably because it is the raw material for all the MBT derivatives.

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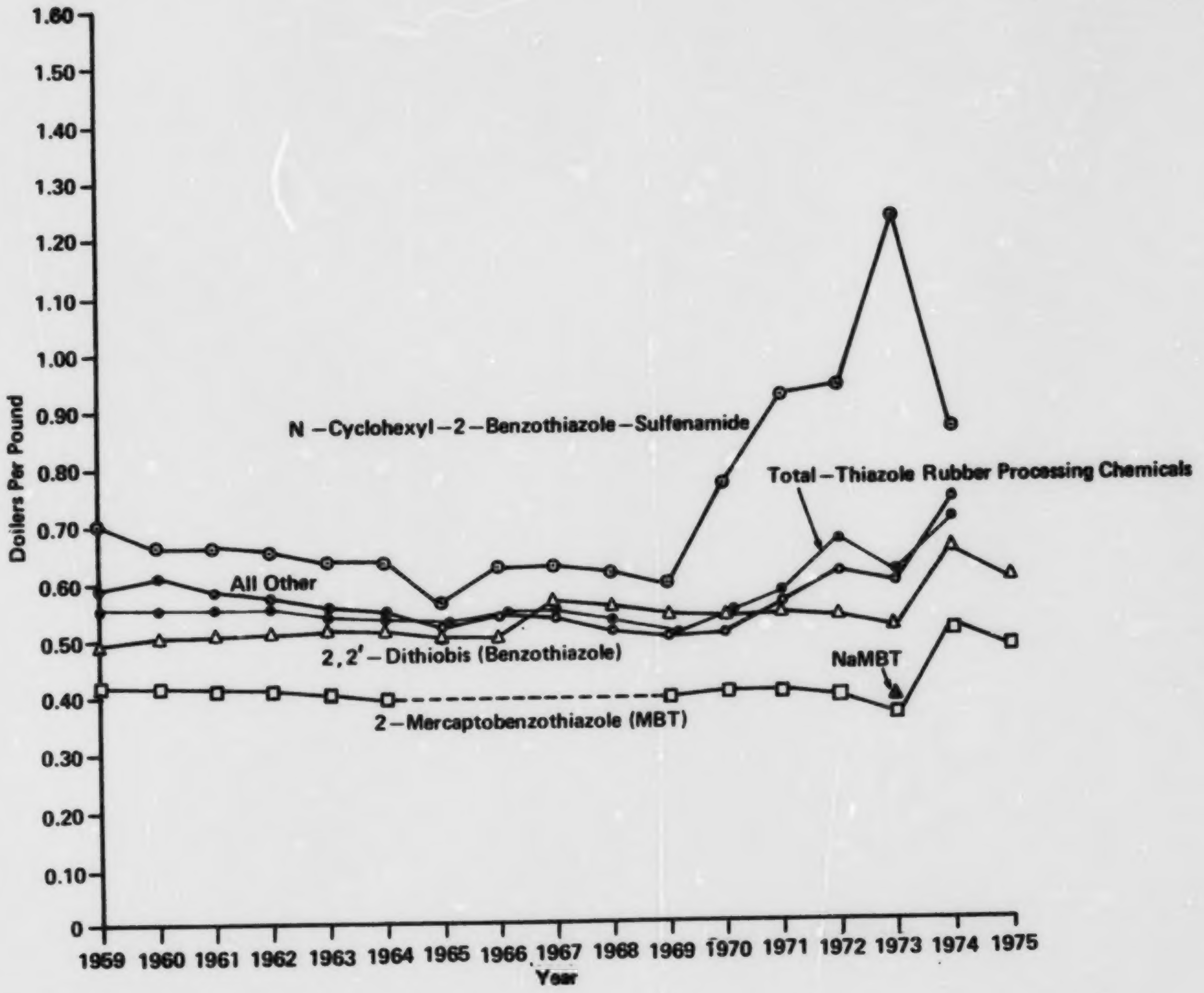

Figure 5. Price History of 2-Mercaptobenzothiazole Derivatives (U.S.I.T.C., 1959-1973; Chemical Marketing Reporter, 1975)

5. Market Trends

Although there is fluctuation in the consumption of mercaptobenzothiazole compounds, the general trend, as shown in Figure 5 and Table 5, is toward increased consumption. The demand for individual members of the thiazole family does not necessarily follow the general trend. The use of MBT has declined since the middle 1960's, while the demand for sulfenamides has increased. MBT has been replaced in formulations with accelerators which approximate its activity, but are less likely to cause scorching (premature vulcanization). As the use of radial tires becomes more widespread, accelerator demand will shift in favor of those compounds which impart the required properties to these tires. In general, the use of radials is expected to decrease the demand for accelerators because of the longer wear expectancy of radial over conventional tires (Anon., 1974).

Since the largest use of MBT compounds is in the rubber industry and the major rubber product is vehicular tires, the mercaptobenzothiazole market is expected to be affected by trends in automobile sales and use. When auto sales are up, tire sales are up. When auto sales are down, as they were in 1974, tire demand does not necessarily decrease since new tires are purchased by consumers to replace worn ones on older vehicles. However, if vehicular use should decline along with auto sales, demand for tires would certainly fall. Such a situation could arise if, for example, gasoline prices were to increase significantly, or in the event of a scarcity and/or rationing of fuel.

The snow tire market also has an impact on overall tire sales. In 1974, General Motors purchased 15 million regular tires for use as original equipment. That same year, 17.5 million snow tires were sold, down from 19.1 million in 1972. In 1975, GM began equipping all new cars with TPC-Spec steel-belted radial tires, which qualify as snow tires in Idaho, Texas, Oklahoma, and New York City. GM has been promoting the TPC-Spec "for year-round traction performance" and this is expected to be a factor in anticipated sales of snow tires below 15 million in 1975 (Anon., 1975a).

The market for mercaptobenzothiazole accelerators is also affected by their cost *vis-à-vis* the costs of competitive chemical accelerators. The prices and availability of raw materials needed to synthesize MBT and its derivatives must therefore be weighed in any market trend speculation. For MBT, the prospects for a steady supply of raw materials at stable prices appear good. In 1973, aniline was in oversupply and at the lowest prices in two decades (Anon., 1973a). There is currently an overcapacity for carbon disulfide, with prices at their highest in 23 years and demand slackening off (due to lower rayon production) (Anon., 1975b). These factors are likely to soften the price of this chemical.

B. Uses

1. Major Uses

Table 7 lists the major and minor uses of MBT and its derivatives. The most important use of these chemicals is as vulcanization accelerators.

Table 7. Use of MBT and Derivatives

MAJOR USES	COMPOUND
Vulcanization Accelerators	all, except NaMBT
Corrosion Inhibitors	NaMBT, MBT
MINOR USES	
Fungicide Formulations	MBT, NaMBT, others
Reagent in Transition Metal Separations and Analyses	MBT, NaMBT

Vulcanization accelerators are selected on the basis of predetermined processing requirements and curing speed (Walker, 1970). A typical rubber compound (mixture of materials prior to vulcanization) contains approximately 0.5 - 1.5% accelerating agent. MBT is the most active of the thiazole accelerators at both processing (mixing, milling, compounding) and curing (vulcanizing) temperatures. It is, therefore, used where high activity is required at relatively low temperatures (below 287°F), and with slower curing synthetic rubbers. MBT is used, for example, in combination with other accelerators in curing butyl rubber and in the manufacture of soles for shoes (Walker, 1970). The combination of MBT and tellurium diethylthiocarbamate is the fastest known accelerator for butyl rubber. It is used extensively for butyl inner tubes in buses (Elkin, 1969).

MBTS is used in curing systems above 287°F. It has less tendency to cause premature vulcanization at higher temperatures than MBT. Its activity can be modified over a wide range when used in combination with MBT and other accelerators.

ZMBT is intermediate in curing rate and tendency to premature vulcanization. It is used in latex foam compounding (Walker, 1970).

As a group, the sulfenamide accelerators have much less tendency to cause premature vulcanization (which leads to a vulcanizate with nonuniform physical properties) than the other thiazole accelerators. Sulfenamides tend to inhibit crosslinking for a time, and then accelerate it (Leib et al., 1970). The first commercial sulfenamide accelerator was N-cyclohexyl-2-benzothiazolesulfenamide (CBS). CBS is approximately equivalent to MBTS in curing properties with somewhat less tendency for premature vulcanization.

N-Oxydiethylene-2-benzothiazolesulfenamide (OBS) is a delayed-action accelerator used with products for which premature vulcanization must be avoided at all costs, and also when high processing temperatures may be encountered prior to curing (Walker, 1970). N,N-Diisopropyl-2-benzothiazolesulfenamide (DIBS) also exhibits the delayed action characteristic of the sulfenamide accelerators. Once activated, DIBS cures very rapidly (American Cyanamid Co., 1973). N-tert-Butyl-2-benzothiazolesulfenamide (TBBS) is a powerful accelerator above 280°F. TBBS gives good protection from premature vulcanization and has somewhat greater cure strength than other sulfenamide accelerators (American Cyanamid Co., 1971).

The largest single product area for vulcanized rubber is tire and tire products for automotive vehicles. In 1972, approximately 1.5 million tons of rubber were used for tire and tire products, accounting for 63% of all rubber production for that year (Oosterhof, 1972). Table 8 shows a passenger tire formulation suggested by R.T. Vanderbilt Company for tire treads. It should be kept in mind that, while this formulation gives typical ingredients and relative quantities, it is not meant to suggest that any particular tire currently manufactured is made from this formulation.

The second major use of MBT compounds, specifically NaMBT, is as a corrosion inhibitor in water based cooling systems, especially those of automobiles. Corrosion in ethylene glycol-water cooling mixtures is due to oxidation of the glycol to organic acids (Collins and Higgins, 1959), which is encouraged by excessive aeration of the antifreeze solution, high temperature sites in the cooling system waterways, or excessively high temperature operation of the engine. It has been shown that in a cooling system made of many types of metals and employing ethylene glycol antifreeze, corrosion activity can be

Table 8. SBR/cis-Polybutadiene Passenger Tire Tread Formulation (Walker, 1970)

	Parts
SBR (styrene-butadiene rubber)	103.1
cis-Polybutadiene	25
K-Stay G (oil soluble sulfonic acid derivative in petroleum base)	5
Stearic Acid	2
Zinc Oxide	3
AgeRite Resin D (polymerized trimethylhydroquinone)	1.5
AgeRite HP (phenyl-β-naphthylamine 65%, diphenyl-p-phenylenediamine 35%)	0.5
Antozite 67S (p-phenylenediamine derivative)	4
Microcrystalline Wax	1
Philrich 5	7
ISAF (intermediate super abrasion furnace black)	65
Sulfur	1.8
Amax (N-oxydiethylene-2-benzothiazolesulfenamide)	1.5
TOTAL	220.4

(Press Cure: 30 minutes @ 307°F)

significantly inhibited by the addition of triethanolamine phosphate and about 0.1% NaMBT (Squires, 1958). The concentration of the NaMBT decreases with service as it tends to form MBTS (Vanderbilt, 1974). The lower limit on concentration for effective corrosion inhibition is about 0.01%. The length of time it takes to reach ineffective levels of NaMBT varies considerably from vehicle to vehicle (Weibull, 1966), hence it is recommended that antifreeze solution should be periodically recharged with a dose of concentrated NaMBT solution (Squires, 1958).

MBT and NaMBT are also being used to protect metals in industrial applications other than automotive. Krapivkina and Rotmistrova (1969) formulated a corrosion inhibiting lubricant containing 1% MBT for use on steel reinforced concrete. The lubricant helped provide protection not only from the weather, but from 6% mineral acids as well.

NaMBT has been shown to restrict corrosion in alcoholic solutions (other than glycols) such as are used for aircraft deicers (Nigam and Sanyal, 1971).

MBT has been recommended to protect copper and brass fittings against attack by chlorinated acetic acid in cellulose processing (Prajapati *et al.*, 1972). In the case of copper and brass, MBT affords protection by inhibiting anodic reactions (Prajapati *et al.*, 1972), whereas the corrosion of steel in sulfuric acid is slowed by MBT mainly by the inhibition of cathodic reactions (Singh and Banerjee, 1972).

MBT can be incorporated into paint formulations intended for electrodeposition on mild steel. The painted steel thereby acquires a very tough finish with high resistance to chipping and flaking as well as corrosion (Guruswamy and Jayakrishnan, 1972).

2. Minor Uses

2-Mercaptobenzothiazole forms characteristic complexes with transition metals (Khullar and Agarwala, 1975) and rare earths (Malik and Rahmani, 1975), and the distinguishing properties of these complexes have been suggested as a means of chemical analysis of the metals. The chemistry of palladium (House and Lau, 1974), platinum, rhodium, iridium (Diamantatos, 1973b), and gold (Diamantatos, 1973a) with MBT has been applied to the development of a solvent extraction separation scheme which offers very high resolution, yet is straightforward enough for routine work in the metals processing industry.

MBT has also been used in metal processing as a collector in flotation liquors. Current applications of MBT include flotation of copper minerals, copper-activated sphalerite, and nickel ores (Jones and Woodcock, 1973).

The benzothiazole moiety is found in some commercially important thiazole and cyanine dyes (Powell, 1969; VanLare, 1965), but these compounds are not manufactured from MBT or MBT derivatives. In fact, although benzothiazole is used somewhat as a dye precursor, it is not used in the above mentioned dyes.

When applied to fabrics, MBT exhibits antifungal properties (Darby and Kempton, 1962; Turner, 1966). MBT is used in leather processing to prevent fungal growth on leather products (Bowes et al., 1970), especially on those meant for tropical use. MBT is also used in antifungal formulations, such as R.T. Vanderbilt Company's Vancide 51, which contains as active ingredients, 27.6% sodium dimethyldithiocarbamate and 2.4% sodium 2-mercaptobenzothiazole (Berg, 1974). Vancide 51 is recommended for a wide variety of antifungal applications: sweet potato seed piece treatment, industrial water cooling slimicide, and as a textile preservative (Berg, 1974). In the case of industrial water cooling systems (water is the heat transfer medium), NaMBT may be present, not only as a slimicide, but also as an anticorrosion agent. Actually, less than 1% of all NaMBT produced is used in antifungal formulations. Most of it is used in anticorrosion applications, with more than 50% of the amount used for anticorrosion being used to protect automobile engines (data provided by industrial sources).

Several halo, alkyl, and thioalkyl derivatives of benzothiazole have shown some anthelmintic or antifungal activity (Alaimo et al., 1974). It is doubtful, however, that these compounds are presently used for these purposes. However, cyanomethylthiobenzothiazole and the monoethanolamine salt of MBT have been produced in commercial quantities for fungicide applications (USITC, 1959-73).

3. Discontinued Uses

MBT causes contact dermatitis in many people. The symptoms are usually produced by intimate contact with an article of clothing made of or containing rubber (Fisher, 1973). Because of this effect, the use of MBT in most Spandex rubber manufactured in the United States for articles of clothing (underwear, girdles, brassieres, etc.) has been discontinued (Jordan, 1972).

There have been shifts in uses of MBT. It is used much less now than formerly as an accelerator in the rubber industry, having been replaced by the sulfenamide derivatives for many applications. It is necessary to synthesize MBT, however, as an intermediate for production of sulfenamide derivatives.

4. Projected or Proposed Uses

MBT compounds will probably continue to be used in novel organic syntheses, as for example in recent studies of β-lactam antibiotics, where the conversion of penicillins into cephalosporins was achieved in reactions which employed MBT as an intermediate reagent (Kamiya et al., 1973). It is difficult to predict whether such uses of MBT will ever be of commercial significance.

5. Possible Alternatives to Use

Although an important commercial chemical with widespread use, MBT could probably be replaced in some applications with other materials. In the case of vulcanization acceleration, there are other types of compounds presently in use, although mercaptobenzothiazoles account for the majority of all accelerator chemicals. Dithiocarbamates and tetra-R-thiouram mono and disulfides are among the "ultra" accelerators presently in use for high temperature and rapid cure vulcanization applications (Walker, 1970). Alone, or in various combinations, these compounds could probably be substituted for mercaptobenzothiazole accelerators in many formulations, although it is possible that other accelerators would be a greater health hazard (e.g., thioureas) than MBT. In some applications, it might be possible to change the type of rubber rather than the type of accelerator in a given rubber. Neoprene rubber, for example, is presently accelerated exclusively with thiourea derivatives, not mercaptobenzothiazoles. (Neoprene rubber represented 6.7% of all rubber types produced in 1972 [SRI, 1972b]).

MBT compounds are only one group of many which are used for the inhibition of corrosion. Substitutes for MBT derivatives include chromates, borates, phosphates, tetraethanolamine, and mixtures of these, as well as sodium benzoate and sodium nitrite. In the case of the cooling systems of automobiles, however, most of the available substitutes would not be as effective as formulations which include MBT (Weibull, 1966). The most effective organic substitute for NaMBT is probably benzotriazole and its sodium salt derivatives. Although currently more expensive than MBT compounds, benzotriazoles are effective at lower concentrations for longer periods of time, and offer superior protection of copper and copper alloys (Davis *et al.*, 1976).

The role of MBT as a fungicide is minor, not only with respect to other uses of MBT, but with respect to all other fungicides as well. There are many possible alternatives to MBT in this application, including copper, mercury, organic and inorganic zinc salts, halogenated phenols, etc. (see, for example, Turner, 1966).

C. Environmental Contamination Potential

1. General

In considering the environmental contamination potential of MBT and its related compounds, it is important to bear in mind that regardless of which of the MBT compounds is employed in a particular use, alteration frequently occurs resulting in the formation of one or more other members of the MBT family. When rubber is vulcanized, for example, a sulfenamide may be the accelerator added during compounding, but due to the chemistry of vulcanization (see Section I-B), one is generally able to detect benzothiazole, MBT, MBTS, and ZMBT (when ZnO is present in the mix) in the vulcanizate. Moreover, mercaptobenzothiazoles present in rubber products usually are oxidized to sulfonates, and eventually benzothiazole, as the rubber ages (Brooks, 1963). In the case of the use of NaMBT in anticorrosion applications, the salt gradually oxidizes to MBTS. It is possible for the NaMBT to undergo reduction as well to benzothiazole (which is insoluble in water) in the presence of iron if the pH of the antifreeze solution falls below 7 (Weibull, 1962).

The most obvious source of environmental contamination potential by MBT compounds, including the parent compound, benzothiazole, is in the use and disposal of rubber tires. The disposal of antifreeze solutions with mercaptobenzothiazole corrosion inhibitors is probably the second largest source of environmental contamination, especially because used antifreeze is likely to be disposed of by simply pouring it on the ground. The compounds in solution can then be spread widely via sewers, ground water, and streams, unlike the case of discarded tires where the chemicals take some time to leach out of the rubber matrix.

2. From Production

MBT is usually manufactured in closed continuous systems which include recovery and recirculation of excess CS_2 (see Figure 4, p. 24). Under normal circumstances, the reactants and products do not have access to the environment. The columns and associated equipment must be able to withstand the high temperatures and pressures required without leaking H_2S, CS_2, or aniline into the environment. Also, there must be provisions for dealing with the H_2S (which is not recirculated), so that it is kept out of air and water.

Manufacturing processes other than the continuous process described in Section II-A-3 (p. 21) may produce side products which are potential environmental contaminants. Ivanova and Shebuev (1957), for example, have detected benzothiazole in the waste water effluent of a MBT manufacturing facility in the Soviet Union.

3. From Transport and Storage

Solid MBT compounds are transported and stored in paper bags and cardboard drums. Generally, the greatest hazard these present is the possibility of dust escaping into the air during filling and emptying procedures, or if accidental breakage should occur. In the event of spills, the recommended cleanup procedure involves the eventual burial of the chemical in landfills or disposal down a sewer (Shaffer, 1971a,b,c,d, 1972a,b,c, 1974), either of which would result in placing the spilled compounds directly in the environment.

NaMBT is usually sold in aqueous solution. It is transported and stored in tanks and drums. Spray from the liquid while being poured, or a leak in a transport pipe are possible sources of contamination with this material. The pH of NaMBT solution is normally quite high (ca. 13, assuming 0.3% free alkali as NaOH) (see Table 4, p. 9), and the solution is therefore a caustic material.

4. From Use

The major environmental contamination potential of MBT compounds is in the use of rubber products. Akron, Ohio, represents the area of the largest concentration of use of vulcanization accelerators in the U.S., but rubber processors are well scattered throughout the U.S. at more than a thousand sites, according to industry sources. Evstifeev *et al*. (1971) have detected MBT and MBTS in the waste effluent of a rubber manufacturing plant in the USSR and in the U.S. Webb *et al*. (1973) have identified MBT and benzothiazole in the holding ponds and aerated lagoons used for treating effluents from synthetic rubber plants. Concentrations of 0.027 ppm and 0.049 ppm benzothiazole have been detected in discharges of a tire plant treatment pond (Niles, 1976).

MBT compounds may be emitted into the air of plants which use these compounds in rubber manufacturing. Evidence of this was shown by Rappaport (1975), who identified the presence of benzothiazole and *t*-butylisothiocyanate in the volatiles of a passenger car tire formulation undergoing vulcanization, which included N-*t*-Butyl-2-benzothiazolesulfenamide as the accelerator. The techniques used were mass spectrometry and gas chromatography. He also found an unusual assortment of other compounds discharged during vulcanization, including styrene, butadiene oligomers, alkyl benzenes, naphthalenes, and some nitrogen and sulfur compounds. The latter nitrogen and sulfur compounds were not attributed to the accelerator. All identified materials appeared to be either impurities in the rubber compound or decomposition products of the compound ingredients.

Approximately 1.2 billion pounds of rubber dust are worn from vehicular tires in the United States each year (Pierson and Brachaczek, 1975). If it is assumed that the accelerators are evenly distributed in this dust, and considering that the tire compound contains about 1% accelerators on the average, roughly 12 million pounds of vulcanization accelerator products, including benzothiazole, ZMBT (providing ZnO is in the formulation), MBT, and MBTS (the exact kinds and quantities of MBT breakdown products from vulcanization has not been determined), get into the air and soil adjacent to highways each year. Since gaseous emission from automobile and truck rubber tires is negligible (Dannis, 1975), airborne particulate debris from tires is likely to be the major source of MBT in the air. Particulate matter from this source, however, constitutes only about 1-4% of all the particulate matter in the air (Pierson and Brachaczek, 1974, 1975) and is, therefore, not a major source of air pollution. Most of the tire dust generated at the road settles in the surrounding area. Under appropriate conditions in moist soil, the rubber particles are degraded within months, thus releasing MBT compounds. Under less favorable conditions, degradation may take decades (Dannis, 1975). The fate of MBT and related compounds in these particles has not been reported, and there is no reason to believe it is currently being investigated. However, it is known that MBT products can easily leach out of rubber which is in contact with water (Aktulga, 1971a). Rubber closures which had been processed with MBTS were used to stopper 500 ml infusion bottles filled with distilled water. When the bottles were inverted and left undisturbed for as little a time as one month, both MBT and MBTS could be identified in the water by both spectrophotometry and thin layer chromatography. Grushevskaya (1974) reported similar results for water shaken with rubber samples. Thin layer chromatography of chloroform extracts of the water demonstrated the presence of MBT, MBTS, and OBS from the rubber in the water.

Consumer exposure to MBT compounds can occur when one wears clothing containing rubber. As noted previously, such contact often leads to dermatitis in individuals sensitive to these chemicals.

Minor potential sources of environmental contamination from uses include antifreeze solutions (see following section) (Squires, 1958), and paints and other materials containing mercaptobenzothiazoles as antifungal agents. This includes certain clothing intended for use in tropical climates.

5. From Disposal

When used tires are discarded in garbage dumps, landfills, or waterways rather than being reprocessed, they become a source of MBT compounds which would be expected to enter the environment readily. It has been shown that MBT compounds easily leach from rubber into stagnant water (Aktulga, 1971a) (see previous section).

A major source of contamination for MBT chemicals is via disposal of waste radiator coolants and antifreeze solutions. These materials are likely to be discarded directly into a sewage system, as compared to rubber tire dust and waste products, from which the accelerators must first leach out Approximately 11 million pounds of NaMBT is produced each year, about half of which goes into antifreeze products which will be discarded within a year or so of their being placed into service.

Excess, contaminated, or surplus MBT compounds, if disposed of by incineration, produce SO_2 (and possibly also CO and nitrogen oxides), and, therefore, mercaptobenzothiazoles disposed of by incineration are probably not a source of MBT contamination. However, many MBT compounds are deposited in landfills (see Section II-D-3, p. 43), and, therefore, leaching from landfills could be a significant source of contamination.

6. Inadvertent Production via Industrial Processes

It is clear from the discussion on vulcanization chemistry that, although a single accelerator compound, say a sulfenamide, may be employed in a given vulcanization operation, the nature of the process is such that a variety of MBT products can usually be identified in the final rubber product, including the one actually added in compounding. It is perhaps not quite correct to say that these other chemicals (usually benzothiazole, MBT, MBTS, and ZMBT) are inadvertently produced, since their formation is an essential part of the vulcanization process. However, since it is not the direct intention of the manufacturer to specifically include these materials in the product, their presence is in that sense inadvertent.

Industrial processes other than vulcanization which might inadvertently produce MBT compounds would have to be characterized by the high temperatures and pressures needed to produce MBT, as well as either the presence of the materials it is made from (aniline, CS_2, and sulfur), or an MBT compound which might be a precursor to other MBT compounds (i.e., a sulfenamide, a metal salt, polysulfide, etc.). It is not likely that dyes containing benzothiazole rings would act as inadvertent precursors to other MBT compounds under the conditions in which thiazole and cyanine dyes are manufactured and used. Phenylisothiocyanate, which is a commercial product (SRI, 1975), could form MBT if it comes in contact with hydrogen sulfide (Ivanova and Shebuev, 1957).

7. Inadvertent Production in the Environment

Except in the depths of volcanoes, the temperatures and pressures needed to produce MBT from its raw materials are not normally encountered in nature. Moreover, it is unlikely that CS_2 and aniline would be present in a volcano, even if sulfur were. Therefore, the possibility of MBT compounds being produced in the environment, even in the presence of all three starting materials, seems very remote.

D. Current Handling Practices and Control Technology

1. Special Handling in Use

Respirators approved by the U.S. Bureau of Mines for nuisance dust and safety spectacles are recommended in the event of excessive dustiness in handling the compounds listed in Table 9. Otherwise, no special handling in use is specified by the manufacturers of these compounds, other than ordinary measures of personal hygiene.

Solutions of NaMBT are alkaline and, therefore, are more dangerous than the solids with respect to skin contact and breathing of any spray from them. Splashproof goggles and protective rubber clothing are recommended for handling such solutions, in addition to the suggestions above. Also, an eyewash fountain and safety shower should be available at the handling site.

2. Methods of Transport and Storage

The solid chemicals listed in Table 9 are supplied in 50 pound paper bags and in 200-250 pound fiber drums. No special storage or shipping methods are used for the solids.

Solutions of NaMBT are shipped and stored in 55 gallon drums and tank cars. The solution is treated with the same precautions as any caustic solution. It otherwise requires no special storage conditions.

3. Disposal Methods

The recommended disposal method for all the compounds listed in Table 9 is burial in a landfill (see references, Table 9). Incineration is not recommended unless provision can be made to insure that SO_2, CO, and nitrogen oxides from the sulfenamides will not be emitted to the atmosphere.

Table 9. Sources of Safety Data for MBT Compounds

Chemical	Safety Reference
2-Mercaptobenzothiazole	Shaffer, 1971d
2,2'-Dithiobisbenzothiazole	Shaffer, 1971a
N-Oxydiethylene-2-benzothiazolesulfenamide	Shaffer, 1974
N-*tert*-Butyl-2-benzothiazolesulfenamide	Shaffer, 1972a
N,N-Diisopropyl-2-benzothiazolesulfenamide	Shaffer, 1972b
N-Cyclohexyl-2-benzothiazolesulfenamide	Shaffer, 1971b
Zinc Mercaptobenzothiazole	Shaffer, 1971c
Sodium Mercaptobenzothiazole, 50% aqueous solution	Shaffer, 1972c

4. Accident Procedures

The solids should be swept up and placed in a waste container and the spill area flushed with water. Rubber gloves and splashproof goggles should be worn to deal with spills of solutions of NaMBT. The solution spill should be covered with a disposable absorbent material, which is then swept up and placed in a disposal container, and the contaminated area should be flushed with water (see references, Table 9).

5. Current Controls and Control Technology Development

MBT compounds are considered relatively free of hazards with respect to fire, reactivity, and the health of humans. The available information indicates that no unusual efforts are made with regard to the manufacturing and handling of these compounds to restrict their entry into the atmosphere as dust or into waste water systems.

E. Monitoring and Analysis

1. Analytical Methods

Until about 15 years ago, colorimetric techniques were recommended for assaying MBT and its derivatives. These are satisfactory for monitoring the purity of individual compounds, but fail to distinguish between the components of a mixture. As a practical matter, it is often important to be able to distinguish MBT from its derivatives, as they will often be found together, as for example, in rubber products, where the addition of MBT and zinc oxide in the compounding stage assures the presence in the vulcanizate of MBT, ZMBT, and also MBTS.

Table 10 summarizes the available analytical methods. Chatterjee et al. (1960) developed a method for the quantitative determination of MBT, ZMBT, and MBTS in the presence of each other via amperometric titration with silver nitrate. The latter forms a silver salt with MBT, but does not react with ZMBT or MBTS. After the determination of MBT, the remaining two compounds are converted, one at a time, into MBT and titrated. This is possible because ZMBT is easily cleaved under acidic conditions, but MBTS requires a reducing agent (Sn^{+2}) and strong acid conditions. The accuracy of this method is better than 2%, typically about 1%, with a detection limit of 0.2% or 2,000 ppm.

Chakravarti and Sircar (1965) extended the above technique to include sulfenamide derivatives of MBT. The sulfenamides are also reduced to MBT in strongly acidic Sn^{+2} solution. They are separated from the other components in the sample by solvent extraction prior to cleavage and titration.

Ethylene glycol antifreeze solutions commonly contain NaMBT as an anticorrosion agent. Usually a mixture of anticorrosion agents is used.

Table 10. Analytical Techniques for MBT Compounds

Technique	Compounds	Sensitivity	Source
Amperometric titration	MBT, ZMBT, MBTS	2000 ppm	Chatterjee *et al.*, 1960
Paper chromatography/ UV-IR spectroscopy	MBT	(qualitative)	Fiorenza *et al.*, 1963
Amperometric titration	thiazole sulfenamides		Chakravarti and Sircar, 1965
Polarography	NaMBT	1 ppm	Woodroffe and Munro, 1970
Gel chromatography	MBT, ZMBT, MBTS		Aktulga, 1971b
Colorimetric method	MBT, MBTS		Stebletsova and Evstifeev, 1971
Mass spectrometry	thiazole sulfenamides	2000 ppm	Hilton and Altenau, 1973
UV spectroscopy	MBT	9-12 ppm	Jones and Woodcock, 1973, 1975
Filtration of air samples, UV spectroscopy	MBT		Krivoruchko, 1972
XAD resin extraction combined with GC-MS	benzothiazole, methylbenzothiazole, and 2-thiomethylbenzothiazole	ppb	Burnham *et al.*, 1973
GC-MS	benzothiazole		Rappaport, 1975
GC	benzothiazole		Parsons and Mitzner, 1975

A typical antifreeze solution can be expected to contain not only NaMBT, but also benzotriazole (triazole, not thiazole), as well as the following ions: benzoate, borate, nitrite, and phosphate (Woodroffe and Munro, 1970). A polarographic technique has been developed for the determination of MBT in corrosion-inhibited glycol mixtures (Woodroffe and Munro, 1970). The method is sensitive to 1 ppm. Benzotriazole may be determined at the same time after separation of the NaMBT on an anion exchange resin.

Paper chromatography coupled with UV and IR spectrophotometry has been used to qualitatively identify MBT in rubber product mixtures (Fiorenza et al., 1963). Gel chromatography has been used for quantitative determination of materials leached from rubber bottle closures (Aktulga, 1971b). The sensitivity of this method, the size of the rubber closures, and the amount of water in the bottles (500 ml capacity) were not given, but quantities of MBT as low as 38 μg were detected, presumably per rubber closure for one month contact of closure and distilled water.

Some monitoring of industrial waste water for MBT and MBTS has been reported in the Soviet Union. One procedure consists of benzene extraction followed by photometric detection (as a cobalt complex). The technique is said to have an accuracy of ±14% and a sensitivity of 5 mg/l (Stebletsova and Evstifeev, 1971).

Krivoruchko (1972) reported a technique for the monitoring of MBT in air. The samples are collected on a filter by aspiration, dissolved in ethanol, and determined spectrophotometrically at 325 nm. The error is claimed to be within 20%.

Hilton and Altenau (1973) have reported a very rapid mass spectrometric procedure for the identification of sulfenamide derivatives of MBT. This method uses small samples of rubber (ca. 0.5 gm) and does not require a solvent extraction step. Low voltage spectra are produced which can accurately distinguish between the various amine moieties of the sulfenamides, thus identifying the original accelerator. The procedure sacrifices sensitivity for speed; its sensitivity is 2,000 ppm. Because the fragment ions typical of the original sulfenamides were not found in this study of rubber samples, the authors concluded that very little unreacted sulfenamide accelerator remains after vulcanization. The amine and benzothiazole products of vulcanization are retained in the rubber matrix either physically or by hydrogen bonding until released by heating or solvent extraction.

Jones and Woodcock (1973, 1975) have developed a method for the determination of MBT in flotation liquors. The technique consists of extraction with chloroform followed by UV spectrometric quantitation. The sensitivity of the method is 9-12 ppm.

Burnham et al. (1973) have used an XAD-2 polystyrene macroreticular resin as an adsorbent to isolate and concentrate organic compounds from water samples. They have isolated and identified compounds using gas chromatography and mass spectrometry (GC-MS) at ppb ranges. Using this technique, they were able to identify benzothiazole, methylbenzothiazole, and 2-thiomethylbenzothiazole in Delaware River water, but the concentrations were not reported.

Rappaport (1975) developed a GC-MS technique which he used to measure volatiles generated by the vulcanization of tire tread stock.

Parsons and Mitzner (1975) were able to detect benzothiazole with a gas chromatographic apparatus developed for analysis of industrial pollutants in stack effluents.

2. Current Monitoring

MBT or related compounds have been found in drinking water and industrial waste water, and tire dust, which will contain MBT vulcanization products, has been found in the air and soil adjacent to highways.

Pierson and Brachaczek (1974, 1975) have demonstrated that 1-4% of all particulate matter in air is debris from tires. Most of the tire dust generated at the road settles on the road or adjacent areas. The particles will degrade at varying rates, depending upon the conditions (Dannis, 1975), and result in possible release of MBT compounds. However, no direct monitoring of MBT compounds in tire dust has been noted.

In 1957, Ivanova and Shebuev (1957) reported the presence of benzothiazole in the waste effluent of a MBT manufacturing plant. Benzothiazole has been noted as a possible contaminant of drinking water (Anon., 1973b). It has been found as a major contaminant, along with a compound identified as "methyl benzothiazole" (probably 2-methylbenzothiazole) and 2-thiomethylbenzothiazole, in water from the Delaware River sampled near an industrial plant in the southern part of Philadelphia (Burnham _et_ _al_., 1973).

The Committee Report on Organic Contaminants in Water of the American Water Works Association (Jenkins _et_ _al_., 1974) identified the compounds listed in Table 11 in finished water. These same compounds also appear in the latest list of organic compounds identified in drinking water which is prepared by the Water Supply Research Laboratory in Cincinnati, Ohio (U.S. EPA, 1975). It is unknown whether these contaminants definitely resulted from commercial MBT compounds.

Table 11. MBT Compounds Identified in Finished Water
(Adapted from Jenkins _et_ _al_., 1974)

As Listed in Jenkins et al., 1974	Probable Standard Chemical Name
benzene thiazole	benzothiazole
methyl benzothiazole	2-methylbenzothiazole
thiomethylbenzothiazole	2-thiomethylbenzothiazole

Evstifeev et al. (1971) have found aniline, CS_2, H_2S, MBT, and MBTS in the waste effluent of a rubber manufacturing plant. The exact nature of the rubber manufacturing processes was not given, nor was any quantitative information on the substances found in the effluent. However, this calls attention to the possibility that other rubber manufacturing facilities may introduce similar chemicals into their waste effluent.

Following toxicity studies on waste water from rubber production as a component of reservoir water, Vaisman et al. (1973) recommended that MBT and MBTS be totally absent from waste water discharged into reservoirs. Webb et al. (1973) found MBT in the aerated lagoon of a synthetic rubber plant and the raw waste of a paper mill. The contents of the former had a disagreeable odor and the contents of the latter were believed to be toxic. However, no quantitative data were available. Rappaport (1975) monitored the air of a rubber passenger tire press room, but was unable to detect benzothiazole, a compound he was able to detect in the air from a simulated vulcanization process. Webb and coworkers (1973) also found "2-benzothiazole" (probably benzothiazole) in a latex plant's holding pond and in a synthetic rubber plant's aerated lagoon. In the former, a concentration of 0.16 mg/ℓ was reported. In addition, concentrations of 0.027 and 0.049 ppm of benzothiazole have been detected in discharges from a tire plant treatment pond (Niles, 1976).

III. Environmental Health Effects

A. Environmental Effects

1. Persistence

a. Biological Degradation, Organisms and Products

Environmental fate of mercaptobenzothiazole and its derivatives has not been investigated. A great majority of these compounds are used in small concentrations as additives to various materials; for example, they are incorporated in rubber, fungicide preparations (e.g., Vancide 51), antifreeze for motor vehicles, etc. Although the degradation of the materials to which they are added has been the subject of many studies, the additive itself has received no attention, presumably because of the low concentrations involved.

The biocidal properties of mercaptobenzothiazole and its derivatives and the nonspecificity of their action (see Owens, 1969; Horsfall, 1956) suggest that they will not be attacked by microorganisms, at least until the concentration has been depleted below critical toxic limits. Mercaptobenzothiazole and its derivatives may combine with Ca^{2+}, Zn^{2+}, or other cations and form insoluble and relatively undissociable salts, which will probably not be easily accessible to the enzyme systems within the microorganisms. Mercaptobenzothiazole derivatives present in vulcanized rubber may not be accessible to microorganisms because of the shielding provided by the rubber matrix.

Thyasen and coworkers (1945) noted that the sulfur in various organic sulfur compounds commonly used in vulcanization processes were not susceptible to oxidation by sulfur oxidizing bacteria which converted the elemental sulfur in rubber to sulfate. The paper does not make it clear, however, whether mercaptobenzothiazole was one of the organic sulfur compounds investigated.

In summary, the biodegradability of MBT and its derivatives is unknown.

b. Chemical Degradation in the Environment

The available information on the chemical reactivity of MBT and MBT derivatives has been reviewed in Section I-B-2 (p. 16). Very little information is available on which estimates of chemical stability in the environment could be based. MBT compounds do not readily hydrolyze or oxidize. They are strong ultraviolet light absorbers at sunlight wavelengths, but no photochemical studies are available. Thus, the chemical stability in the environment is unknown.

2. Environmental Transport

No experimental work has been reported concerning the environmental transport and behavior of mercaptobenzothiazole and its derivatives. From the chemical and physical properties of these compounds, we have attempted to derive some theoretical information on their environmental transport and behavior. Mercaptobenzothiazoles are, in general, nonvolatile, and thus, it can be suggested that they will enter and/or distribute in the atmosphere to only a small extent.

Sodium mercaptobenzothiazole, which is used as an anticorrosive agent in antifreeze for automobiles, may be converted to relatively insoluble salts of Ca, Zn, etc. upon release to the environment, and thus, the rate of transport to the atmosphere will be significantly reduced (due to increased molecular weight, lower volatility, and decreased solubility). At low environmental concentrations, mercaptobenzothiazoles may be soluble enough to migrate through soil and eventually make their way to ground water. In fact, Aktulga (1971a, b) has demonstrated that MBT is soluble enough to be leached out of rubber with distilled water.

3. Bioaccumulation and Biomagnification

Bioaccumulation of a chemical occurs when the chemical is taken in by an organism faster than it is eliminated. Bioaccumulation potential of a chemical in an organism can be assessed sometimes from the partition coefficient of the chemical. Neely and coworkers (1974) have reported a linear relationship between octanol-water partition coefficients and bioconcentration of chemicals in trout muscle (ratio of the concentration of chemical between trout muscle and the exposure water measured at equilibrium). Unfortunately, octanol-water partition coefficients for mercaptobenzothiazoles are not available in the literature. For benzothiazole (partition coefficient, 2.02), the bioconcentration factor is calculated to be 16. Since the substitution of the mercapto group on benzothiazole should increase its water solubility and reduce the lipid solubility, the bioconcentration factor for mercaptobenzothiazoles will probably be somewhat lower. Salts of mercaptobenzothiazole (calcium, zinc mercaptobenzothiazoles, etc.) will be expected to have even a lower solubility in lipids and, therefore, it is anticipated that they will bioaccumulate to even a lesser extent. Overall, it appears unlikely that mercaptobenzothiazoles and their salts will bioaccumulate to a significant extent in organisms.

Biomagnification refers to a chemical's movement through the food chain resulting in an increase in concentration at each trophic level. In the absence of the experimental data on biomagnification potential of mercaptobenzothiazoles, an attempt has been made to predict the biomagnification potential from the water solubility data. Metcalf and Lu (1973) have found that the ecological magnification in fish of the chemicals studied in their model ecosystem, followed a straight line relationship with water solubility. Using this relationship, the ecological magnification for mercaptobenzothiazole is calculated to be in the range of 60-70 (for comparison, that of DDT is approximately 170,000). From this, it appears that some biomagnification of mercaptobenzothiazole is possible in the environment, although biomagnification to hazardous levels seems unlikely.

B. Biological Effects

1. Toxicity and Clinical Studies in Man

The history of human experience with the adverse effects resulting from exposure to MBT and its derivatives is an account which details numerous cases of allergic contact dermatitis as the only consequence of exposure. Several retrospective investigations have established that MBT, particularly as a component in rubber products, is one of the most common contact allergens known today.

Allergic contact dermatitis is a delayed hypersensitivity reaction that results from exposure of previously sensitized individuals to an allergenic chemical or substance. The induction or incubation phase of contact hypersensitivity begins with the initial exposure to a contact allergen when it forms an antigenic hapten-carrier complex with epidermal proteins (Eisen _et_ _al_., 1952; Eisen and Tabachnick, 1958; Nakagawa _et_ _al_., 1971). Sensitization then takes place in the regional lymph nodes when the antigenic substance combines with receptor molecules on the surface of lymphocytes (Gell and Godfrey, 1974). Sensitized lymphocytes become blast cells and subsequently enter the circulation and bone marrow to function as memory cells in maintaining the state of contact sensitivity (Schneider, 1974). The incubation period may last from 5 to 21 days and may be followed by a "spontaneous" flare-up reaction at the site of exposure, due to minute concentrations of the allergen which may be remaining in the now sensitized skin. Re-exposure to the allergen after the incubation period will cause clinical symptoms of dermatitis which include redness, edema, papules, vesiculation, weeping of the skin, and pruritis (Fisher, 1973). Delayed contact hypersensitivity reactions involve only the cell-mediated immune system; humoral antibodies do not participate in the response to contact allergens (Catalona et al., 1972). Primary irritant dermatitis is distinguished from allergic contact dermatitis in that skin lesions are produced upon the initial application of the irritating substance, and a state of hypersensitivity is not induced.

An indication of the importance of the MBT derivatives as allergenic components of rubber is offered by Fisher (1973) who concluded that the five most common causes of allergic contact dermatitis in order of frequency are as follows:

1. Rhus (poison ivy, oak or sumac)
2. p-Phenylenediamine
3. Nickel compounds
4. Rubber compounds
5. Ethylenediamine

The following sections will discuss the large body of evidence which has accumulated indicating that MBT and several related compounds are a significant cause of allergic contact dermatitis in human populations. In addition, the results of numerous animal studies involving various substituted benzothiazoles will be presented in subsequent sections to demonstrate the varied and profound pharmacologic actions of this class of compounds, which result from simple molecular modifications of the basic benzothiazole structure.

a. Epidemiologic and Controlled Human Studies

The potential for human exposure among the general population to MBT derivatives is extremely high, based on their widespread use as components of rubber articles. Several studies have been undertaken to clearly define the role of benzothiazole derivatives as causative agents in cases of contact dermatitis.

In an early study, Bonnevie and Marcussen (1944) investigated 74 cases of eczema caused by exposure to rubber. Among these patients, 53 (72%) reacted positively to MBT when it was applied to the skin.

Routine patch-testing for sensitivity to rubber chemicals was conducted on a group of 401 patients who were affected by dermatitis of the hands and/or feet (Gaul, 1957). Among these, 5 females and 6 males reacted positively to MBT.

Blank and Miller (1952) tested rubber adhesives in shoes as the cause of dermatitis of the feet among a group of 24 patients. In each of these cases, the wearing of a particular pair of shoes could be correlated to the history of dermatitis. Patch tests were performed using 10 representative antioxidants and 17 accelerators used by the rubber industry. These materials were tested by application of a 1% concentration in petrolatum of each chemical to the upper arm, left on the skin for 48 hours. Reactions were read one hour after removal of the patch, and only vesicular reactions were recorded as positive. Their results for 21 of these patients are summarized in Table 12. A summary of the patch test data as presented in Table 13 clearly reveals that more than half the patients who displayed positive reactions to MBT were also sensitive to a number of other MBT type compounds. These data suggest that cross-sensitization may commonly occur among compounds containing the mercaptobenzothiazole moiety:

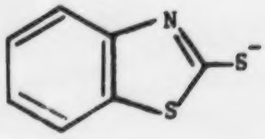

Table 12. Patch Test Reactions to Accelerators and Antioxidants* (from Blank and Miller, 1952)

	Group I										Group II			Group III				Group IV			
Case number	1†	2†	3	4	5	6	7	8	9	10	11	12	13	14†	15†	16	17	18	19	20	21
Accelerators																					
Mercaptobenzothiazole type																					
2-Mercaptobenzothiazole	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	+	-
2,2'-Benzothiazyl disulfide	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	-	-
Zinc benzothiazyl sulfide	0	-	-	-	0	0	-	0	0	0	+	+	+	-	0	0	-	0	0	0	0
Benzothiazyl sulfenamide	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	-	-
N-Anilinomethyl-2(3)-benzothiazolethione	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+	+	-	+
Mixed																					
Zinc benzothiazyl sulfide and tetramethylthiuram monosulfide	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	-	-
A mercaptobenzothiazole and a dithiocarbamate	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	-	-
Dithiocarbamyl type																					
Tetramethylthiuram monosulfide	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-
Tetramethylthiuram disulfide	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-
Selenium dimethyl dithiocarbamate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
N-pentamethylene ammonium pentamethylene dithiocarbamate	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Miscellaneous																					
2-Mercaptothiazoline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diphenylguanidine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diphenylguanidine phthalate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Butylaldehyde-aniline condensation product	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Triethyltrimethylenetriamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexamethylene tetramine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antioxidants																					
Monobenzyl ether or hydroquinone	+	+	-	+	+	+	0	0	0	0	-	-	-	+	+	+	+	+	+	+	+
S-di (β-naphthyl)-p-phenylenediamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mono and dioctyl diphenylamines (mixture)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polymerized trimethyl dihydroquinoline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Aldol alpha naphthylamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenyl beta naphthylamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenyl alpha naphthylamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Reaction product of diphenylamine and acetone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
A diarylamine-ketone-aldehyde reaction product and N,N'-diphenyl-p-phenylenediamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetone and aniline condensation product	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyalkalated phenol sulfide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*- = no reaction or mild erythema; + = vesiculation, 0 = not tested.

† These patients were tested with special shoe linings (see text).

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Table 13. **Summary of Patch Test Data* (from Blank and Miller, 1952)**

Case No.	Shoes	Mono-benzyl Ether of Hydro-quinone	Mercapto-benzo-thiazole Type	Dithio-carbamyl Type	Others
Group I					
1†	+	+	-	-	-
2†	+	+	-	-	-
3	+	+	-	-	-
4	+	+	-	-	-
5	0	+	-	-	-
6	0	+	-	-	-
7	+	0	-	-	-
8	+	0	-	-	-
9	+	0	-	-	-
10	+	0	-	-	-
Group II					
11	+	-	+ (7)	-	-
12	+	-	+ (7)	-	-
13	+	-	+ (5)	+ (5)	-
Group III					
14†	+	+	+ (1)	-	-
15†	+	+	+ (6)	-	-
16	0	+	-	+ (2)	-
17	+	+	-	+ (4)	-
Group IV					
18	-	0	+ (6)	-	-
19	+	0	+ (6)	-	-
20	+	0	+ (1)	-	+ (1)
21	+	0	+ (1)	-	+ (2)
Group V					
22	+	-	-	-	-
23	+	-	-	-	-
24	+	-	-	-	-

---

*- = no reaction or mild erythema; + = vesiculation; 0 = not tested
Numbers in parentheses indicate the number of accelerators of the type indicated to which the patient reacted.

† These patients were tested with special shoe linings.

The problem of shoe dermatitis and its relationship to allergenic chemicals was further studied by Cronin (1966). During the 13 year period between 1953 and 1966, a total of 100 patients were seen with dermatitis of the feet caused by allergic reactions to rubber. Of this group, 68 were women and 32 were men, with their ages ranging from 3 to 75 years. Patch tests were conducted with pieces of the patient's shoe which corresponded to the areas of dermatitis and with the rubber chemicals MBT and tetramethylthiuram disulfide. Of the 100 patients tested, 45% were sensitive to MBT, 12% were sensitive to tetramethylthiuram disulfide, and 37% were sensitive to both. Only 2 patients in this group reacted to rubber from their shoes without also reacting to one or both of these rubber chemicals.

A more extensive investigation of dermatitis caused by rubber was conducted by Wilson (1969) and involved the cases of 106 patients seen from 1955 to 1967 who developed dermatitis from specific rubber articles such as household gloves, condoms, footwear, and girdles. These patients, 76 women and 30 men, developed sensitivity reactions to rubber at various ages (Figure 6) and presented a length of history which varied from one or two weeks to as long as 30 years.

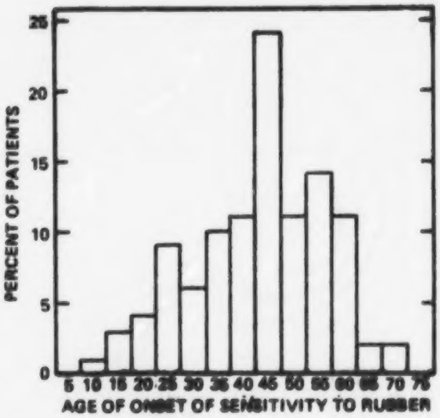


Figure 6. Distribution in Age of Onset of Rubber Sensitivity (from Wilson, 1969)

Seventy patients were sensitized by specific rubber articles; 43 to gloves, 9 to boots and shoes, 9 to condoms, 7 to girdles, brassieres and suspenders, and one each to bathing hats and elastic bands. The remaining 36 patients developed dermatitis from more than one rubber article and it was uncertain which was originally responsible. These patients were grouped according to the article which produced dermatitis and were then patch tested against several rubber accelerators. The substances tested were dipentamethylenethiuram disulfide (PTD), tetramethylthiuram disulfide (TMT), MBT, and zinc diethyldithiocarbamate (ZDC). All chemicals were applied to the skin as a 1% concentration in soft paraffin and read after 48 hours. The results of these tests are presented in Table 14. Forty-five patients reacted to a single chemical only; 26 to MBT and 19 to PTD. In addition, 8 patients reacted to MBT, PTD, and TMT. All 9 patients with dermatitis from boots were reactive to MBT. Sixty-eight percent of the tests were positive to PTD, 54% to TMT, 47% to MBT and 24% to ZDC.

In other studies on dermatitis associated with specific rubber articles, a large percentage of allergic dermatitis cases have been associated with MBT. In a study of shoe dermatitis, Calnan and Sarkany (1959) presented 37 rubber sensitive patients, 19 of whom reacted to MBT. Hindson (1966) studied 30 patients that reacted to rubber condoms. Of these, 6 were found to react to MBT.

A major survey of contact allergens in the United States has recently been conducted (Baer et al., 1973) which provides convincing evidence that MBT is indeed one of the most common and effective dermatitis-producing substances in use today. This report listed contact allergens which

Table 14. Patch Tests with Rubber Accelerators (from Wilson, 1969)

		Gloves		Boots		Condoms		Girdles		Mixed		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
PTD	+	34	79	3	33	9	100	4	57	23	70	73	68
	-	9	21	6	66	0	0	3	43	13	30	31	31
	Total	43		9		9		7		36		104	
TMT	+	19	45	2	22	4	44	6	100	22	61	53	52
	-	23	55	7	77	5	55	0	0	14	39	49	48
	Total	42		9		9		6		36		102	
MBT	+	13	30	9	100	1	11	4	66	19	53	46	45
	-	29	70	0	0	8	89	2	33.	17	47	56	55
	Total	42		9		9		6		36		102	
ZDC	+	4	15	2	33	1	17	3	75	7	23	17	24
	-	22	85	4	66	5	83	1	25	23	77	55	76
	Total	26		6		6		4		30		72	

elicited positive reactions in more than 3% of 200 or more patients tested over the period from 1968 to 1970. Almost all test subjects were suffering from some type of skin disease, and, therefore, the subjects were a selected group rather than a representative sample of the general population. In the case of MBT and several other chemicals, two groups of patients were selected before testing. One group was suspected of having contact dermatitis due to shoes or rubber, and, therefore, that group was tested with the "shoe and rubber tray." The second group displayed clinical signs and histories which did not specifically indicate shoe or rubber allergy, and, therefore, the group was tested with the "diagnostic tray." Table 15 presents the percent of patients which reacted to each of the allergens tested. In terms of overall number of reactions, MBT ranked second among the 24 most common contact allergens. The intensity of reactions to the allergens tested ranged from weak (1+ or 2+) to moderate (3+) and severe (4+). The distribution of intensities of skin reactions elicited with the contact allergens is presented in Table 16.

Cross-Sensitivity

With the high incidence of allergic skin reactions due to MBT exposure having been established by the studies discussed above, the importance of cross-sensitivity reactions to other benzothiazole derivatives is clearly a significant problem. Fregert (1969) has undertaken to identify cross-reacting substances in MBT-sensitized patients. Cross-sensitivity patterns were investigated in 12 patients, 8 women and 4 men, with contact sensitivity to rubber gloves. Allergy to MBT was established by patch testing with 2% commercial grade MBT in petrolatum. Eleven test substances, listed in Table 17, were applied in a 2% concentration to the back of each patient for 48 hours, and the reaction

Table 15. Percent of Patients Reacting to Contact Allergens (from Baer *et al.*, 1973)

Allergens	No. of Patients Tested	% Reacting
Mercury bichloride, 0.05% aqueous solution	540	22.2
Mercaptobenzothiazole, 1% in petrolatum		
Shoe and rubber tray	229	18.8
Diagnostic tray	540	7.8
Paraphenylenediamine, 2% in petrolatum		
Diagnostic tray	540	13.5
Shoe and rubber tray	229	7.0
Potassium dichromate, 0.5% aqueous solution		
Shoe and rubber tray	229	13.5
Diagnostic tray	540	9.8
Ethylenediamine, 1% in petrolatum	158	13.2
Nickel sulfate, 5% aqueous solution		
Diagnostic tray	540	13.1
Shoe and rubber tray	229	8.3
Turpentine, 10% in olive oil	540	12.2
2% aqueous formaldehyde solution		
Shoe and rubber tray	229	11.8
Diagnostic tray	540	8.7
Poison ivy oleoresin, in acetone	340	11.2
Bismarck brown	229	10.5
Thiram, 1% in petrolatum	229	7.9
Diphenylguanidine, 0.5% in petrolatum	229	7.9
Peruvian balsam, 25% in petrolatum	340	7.9
Resorcin, 5% aqueous solution	340	7.9
Monobenzone, 2.5% in petrolatum		
Shoe and rubber tray	229	7.0
Diagnostic tray	540	2.6
Epoxy resin, 1% in petrolatum	340	5.6
Ethylaminobenzoate, 5% in petrolatum	540	5.2
Neomycin sulfate, 20% aqueous solution	540	5.2
Chrysoidine brown	229	4.8
Epoxy hardener, 1% in petrolatum	340	4.4
Acrylic monomer, 25% in olive oil	340	4.4
2-naphthyl benzoate, 1% in petrolatum	229	3.5
Hexachlorophene, 1% in petrolatum	340	3.2
Pyrethrum	540	3.1

Table 16. Degree of Reaction to Contact Allergens (from Baer *et al.*, 1973)

	No. of Patients Reacting			
Allergens	1+	2+	3+	4+
Mercury bichloride, 0.05% aqueous solution	65	32	23	0
Mercaptobenzothiazole, 1% in petrolatum				
Shoe and rubber tray	10	11	18	4
Diagnostic tray	8	20	13	1
Paraphenylenediamine, 2% in petrolatum				
Diagnostic tray	12	24	26	11
Shoe and rubber tray	1	3	12	0
Potassium dichromate, 0.5% aqueous solution				
Shoe and rubber tray	8	10	10	3
Diagnostic tray	15	18	18	2
Ethylenediamine, 1% in petrolatum	1	4	12	4
Nickel sulfate, 5% aqueous solution				
Diagnostic tray	16	13	31	11
Shoe and rubber tray	3	7	6	3
Turpentine, 10% in olive oil	29	21	16	0
2% aqueous formaldehyde solution				
Shoe and rubber tray	16	5	5	1
Diagnostic tray	28	12	6	1
Poison ivy oleoresin in acetone	9	6	19	4
Bismarck brown	19	4	1	0
Thiram, 1% in petrolatum	5	2	8	3
Diphenylguanidine, 0.5% in petrolatum	12	5	1	0
Peruvian balsam, 25% in petrolatum	13	12	2	0
Resorcin, 5% aqueous solution	15	8	3	1
Monobenzone, 2.5% in petrolatum				
Shoe and rubber tray	10	5	1	0
Diagnostic tray	8	5	1	0
Epoxy resin, 1% in petrolatum	5	8	6	0
Ethylaminobenzoate, 5% in petrolatum	9	10	8	1
Neomycin sulfate 20% aqueous solution	6	10	8	4
Chrysoidine brown	2	5	4	0
Epoxy hardener, 1% in petrolatum	6	5	4	0
Acrylic monomer, 25% in olive oil	9	2	3	1
2-naphthyl benzoate, 1% in petrolatum	2	2	4	0
Hexachlorophene, 1% in petrolatum	4	5	2	0
Pyrethrum	7	7	2	1

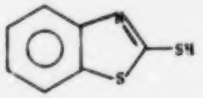
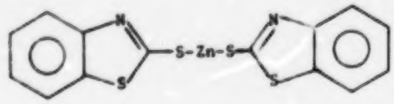
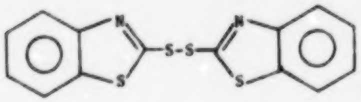
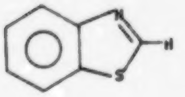
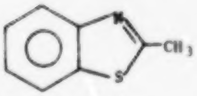
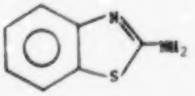
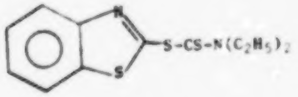
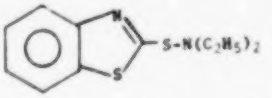
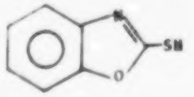
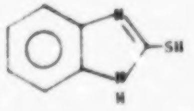
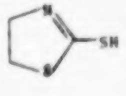
was read 24 hours later. A reaction producing infiltration and/or vesicles or papules was scored as positive. Each of the test substances was also applied in the same manner to non-sensitized control subjects as a test for primary irritant action. All the controls produced negative results. Results of the patch tests for cross-sensitivity are presented in Table 18. Four chemically related MBT derivatives produced positive reactions in all of the patients tested. No variations were observed in this pattern of sensitivity.

An examination of the results presented in Table 18 indicates the importance of both the thiol group in the 2-position and the integrity of the benzothiazole structure as determinants in producing cross-sensitizing reactions to MBT.

Table 17. Test Substances and Their Occurrences (from Fregert, 1969)

1. 2-Mercaptobenzothiazole; used as rubber accelerator and retarder as well as anti-rust agent.
   Trade names: Vulcafor MBT, Merkapto, Rotex, Captax, MBT, etc.
2. Zinc-2-mercaptobenzothiazole; used as rubber accelerator and antioxidant.
   Trade names: Bantex, MBT, Vulkacit ZM, Zenite special, Zetax, etc.
3. Di-benzothiazyl-disulphide; 2,2'-dithiobisbenzothiazole; used as a rubber accelerator and retarder.
   Trade names: Altax, MBTS, Vulcafor MBTS, Vulcacit DM, etc.
4. Benzothiazole; not used in rubber.
5. 2-Methylbenzothiazole; not used in rubber.
6. 2-Aminobenzothiazole; not used in rubber.
7. 2-Benzothiazyl-N,N-diethylthiocarbamyl sulfide; used as rubber accelerator.
   Trade name: Ethylac
8. N,N-Diethyl-2-benzothiazolesulfenamide; used as rubber accelerator.
   Trade name: Vulkazit AZ
9. 2-Mercaptobenzoxazole; not used in rubber.
10. 2-Mercaptobenzimidazole; ethylene-thiourea; used as rubber accelerator.
    Trade names: Na-22, Vulkacit NPV
11. 2-Mercaptothiazoline; used as rubber accelerator.
    Trade name: Accelerator 2-MT

Table 18. Patch Test Reactions in Twelve Patients (from Fregert, 1969)

Substance No.	Formula	Reaction
1		+
2		+
3		+
4		—
5		—
6		—
7	 N S S-CS-N(C2H5)2	+
8		+
9		—
10		—
11		—

Human Assay of Contact Allergens

A considerable amount of work has been performed in order to improve the standard methods of detecting contact allergens. Kligman (1966a) has pointed out that major deficiencies exist in the standard predictive tests used to assay moderately strong contact allergens. Notably, retrospective testing of known allergens resulted in high insensitivity to substances of weak and moderate sensitizing capabilities. These testing methods, therefore, would be expected to fail as a means of screening potential allergens of moderate strength.

As an alternative means of identifying contact allergens by human assay, Kligman (1966b,c) has developed specialized procedures designed to be free of producing false negative results. This so-called "maximization test" involves an induction phase whereby the test site is treated 24 hours with an occlusive patch of 1.0 ml of 5% aqueous sodium lauryl sulfate (SLS). This procedure causes a mild inflammatory reaction which promotes sensitization. To the same site, a 48 hour occlusive patch is then applied which contains the test material. This sequence is repeated five times, alternating 24 hour irritant patches and 48 hour allergen patches. Following the induction period, a challenge test is performed which consists of pre-treating the skin occlusively with 0.4 ml of 10% SLS for one hour and followed by occlusive application of the test substance for 48 hours. This procedure is referred to as the "SLS provocative test." When MBT was tested on healthy adult subjects according to Kligman's maximization procedure, a high rate of sensitization was achieved. Table 19 lists these results together with those obtained by testing of various industrial chemicals. The sensitization grade of 3 for MBT indicates that a moderate skin reaction was

Table 19. Industrial Contactants (from Kligman, 1966c)

	Induction concentration (%)*	Challenge concentration (%)**	Sensitization rate	Sensitization grade
Benzene	50	20	0/25	1
Xylene	100	25	0/24	1
Pyridine	50	10	1/24	1
Dimethylsulfoxide (DMSO)	75	25	0/23	1
Hexane	100	25	0/25	1
Chloronaphthalene	25	10	0/25	1
Aniline	20	10	7/25	2
Mercaptobenzothiazole	25*	10**	9/24	3
Nickel sulfate	10	2.5	12/25	3
Chromium trioxide	3	0.5	13/23	3
Chromium sulfate	25	2.0	11/23	3
Cobaltous sulfate	25	2.5	10/25	4
Gold chloride	2	0.005	16/23	4
Turpentine	50	20	18/25	4
Butylglycidyl ether	10	10	19/24	4
Beryllium sulfate	5	1.0	18/22	4
2-amino-5-diethylaminotoluene HCl	25*	10**	19/25	4
Potassium dichromate	2	0.25	23/23	5
Phenyl mercuric nitrate	2	0.5	24/25	5
Thioglycerol	50	5	24/24	5
Diethylenetriamine	10	10	24/25	5
Diethylfumarate	1.0	0.2	25/25	5
n-Butylthiomalate	5.0	1.0	22/25	5
Technical Malathion ®	25*	10**	25/25	5
Glyoxal	10	2.0	24/24	5
Krameria (Extract)	25*	10**	22/22	5
Expoxy resin	25	15	21/25	5
Hydrazine	5	0.5	23/23	5

* Pre-treatment of skin with SLS

** SLS provocative test

produced. The reaction grading was placed on a scale from 1 to 5 with increasing numbers corresponding to weak, mild, moderate, strong and extreme reactions, respectively. Kligman (1966c) noted that 90% of the test subjects used in this study were Negroes, who are less susceptible to inflammatory skin reactions. The author predicted, therefore, that an even higher rate of sensitization might be achieved among white subjects using the same test procedures.

b. Occupational Studies

There has been comparatively little evidence of occupational disease which can be attributed solely to contact with MBT or its derivatives. Undoubtedly, much of the reason for the lack of occupational poisonings is due to modern hygiene practices and well-equipped manufacturing facilities. Nevertheless, several surveys of health problems in the rubber industry have revealed that occupational diseases are not uncommon, and MBT derivatives may very well play a role in contributing to adverse health effects.

In a report on allergy in the rubber industry, Wilson et al. (1959) noted that occupational dermatitis was a common occurrence, being referred to as "rubber itch" and "rubber poisoning." At that time, contact with chemicals was regarded as being responsible for 90% of all occupational allergies in the rubber industry. These chemicals would include, in addition to the benzothiazole-type accelerators, a number of antioxidants, softening oils, phenol-formaldehyde resins and epoxy resins.

A study which demonstrated that MBT was specifically responsible for causing occupational dermatitis has been conducted by Herrmann and Schulz (1960). Between 1953 and 1958, 8,000 patients with contact dermatitis were seen, among which 63 cases were due to rubber. Of these, 29 were occupational in origin, and 16 occurred in the rubber industry. Patch testing was conducted on these 16 patients with a number of industrial and rubber chemicals to determine the specific allergen involved (Table 20). In most cases, derivatives of MBT were the most common causative agents.

In a recent European study (Oleffe et al., 1972) 300 patients suspected of having occupational contact dermatitis were exposed by patch tests to 20 allergenic chemicals, including MBT. The group consisted of 184 men (61%) and 116 women (39%). Among these patients, 4 men (2.2%) and 5 women (4.3%) displayed positive reactions to MBT. With men and women combined the number of persons with positive reactions to MBT was 3% of the total number of subjects.

Product safety data sheets supplied by Uniroyal Chemical (Uniroyal Chemical, 1971a,b,c,d) provide statements of the medical history in the manufacture of several MBT derivatives. A record of no adverse history based on over 20 years of production and use was claimed for 2,2'-dithiobisbenzothiazole and zinc MBT. No history of medical problems was encountered based on several years of production and use of N-cyclohexyl-2-benzothiazolesulfenamide and N-*tert*-butyl-2-benzothiazolesulfenamide.

c. Non-Occupational Exposures and Poisoning Incidents

The great majority of exposures in the general population to derivatives of MBT undoubtedly occur by contact with clothing and rubber articles which contain these substances. Reported incidents of adverse effects resulting from such contact have been limited exclusively to the development of allergic contact dermatitis. Individual cases have involved dermatitis resulting

Table 20. Positive Skin Reactions in 16 Patients with Rubber Allergy (data from Herrmann and Schulz, 1960)

Substance	No. of positive reactions
2-Mercaptobenzothiazole (MBT)	10
2,2'-Dithiobisbenzothiazole (MBTS)	4
N-Cyclohexyl-2-benzothiazolesulfenamide (CBS)	6
Mixture containing:	
72% MBTS	
13% Diphenylguanidine	5
15% Hexamethylenetetramine	
Tetramethylthiuram disulfide	4
Diphenylguanidine	6
o-Tolylbiguanidine	5
Hexamehylene tetramine	-
p-Phenylenediamine	3
p-Nitrosodimethylaniline	-
N-Phenyl-N'-cyclohexyl-p-phenylenediamine	3
Phenyl-β-naphthylamine	2
Condensation product of acetaldehyde and α-naphthylamine	5
4,4'-Dioxydiphenyl	2

from rubber accelerators in shoes and sneakers, bathing caps, condoms, gloves and rubber in elastic linings (Jordan, 1972).

Women are considered to be particularly vulnerable to MBT-induced allergies due to their high degree of exposure to items containing rubber. Bauer (1972) reported that MBT may cause dermatitis in women who are in contact with it as a component in girdles, brassieres, "falsies," elastic in underpants and hair nets, boots, shoes, and dress shields.

Porter and Sommer (1967) described the cases of 5 women who developed allergic contact dermatitis which corresponded to certain portions of their brassieres. Patch tests were performed on each patient using various substances including 1% MBT in petrolatum. In every case the patients were sensitive to MBT and to spandex, a polyurethane elastomer used in making brassieres.

A case of severe contact dermatitis was presented recently by Epstein (1973). In this incident, a 14 year old boy developed severe dermatitis on his feet which spread within a few months' time to involve his hands, forearms, and legs as well. Two years later, after numerous treatments had failed to improve his condition, the boy developed contact dermatitis under the elastic waistband of a new pair of undershorts. Subsequent patch testing revealed a positive sensitivity to the elastic waistband, his sneakers, another pair of shoes, and MBT. The avoidance of shoes containing rubber caused a dramatic improvement in his condition. Although severe reactions to rubber, and MBT in particular, are not very common, this case emphasizes the importance of proper diagnosis in recognizing a potentially dangerous allergy to MBT.

The use of rubber products in the course of dental practice has resulted in rubber dermatitis in the form of contact stomatitis in the mouth.

Everett and Hice (1974) have presented the case of a 16 year old girl with full orthodontic appliances, including intermaxillary rubber bands, who developed an acute redness and swelling of both checks. Prompt recovery was achieved when the rubber bands were removed from her mouth.

The literature has clearly indicated that MBT and other related rubber accelerator chemicals are a threat to the general public insofar as the development of contact dermatitis is concerned. Reports have not been encountered, however, which offer any evidence whatsoever that benzothiazole derivatives have caused a single human fatality or have been identified as the causative agent of a disease other than allergic contact dermatitis. This observation in itself is remarkable in light of the data presented in reports of various animal studies (see following sections) which demonstrate that many benzothiazole derivatives elicit potent pharmacological effects. It should be noted, however, that potential environmental exposures to MBT and its derivatives are not likely to be sufficiently high to produce dramatic symptoms of acute toxicity.

2. Effects on Non-Human Mammals

A sizable body of data exists describing the abundance of physiologic actions that are produced by the introduction of benzothiazole and its derivatives into different animal systems. While many of these compounds are not commercially important and present little environmental contamination potential per se, they are nevertheless good examples of highly active substances which can be derived by simple chemical substitution. Therefore, biological data will be presented and discussed where the activity of a specific substituted benzothiazole may be indicative of the properties of an entire group for which data is lacking.

Furthermore, it is not unreasonable to assume that highly toxic, but commercially unimportant, benzothiazole derivatives may be produced from less toxic but more abundant substances by reaction with other environmental chemicals. In addition, biological and non-biological oxidation, reduction and hydrolysis reactions can significantly alter the form of a substance to which organisms are exposed. Therefore, a cautious extrapolation of the data presented in the following sections will, in many cases, be the best means presently available to predict the potential harm that may result from indiscriminate exposure to these substances.

a. Absorption Studies

Specific studies have not been encountered which measured either the routes or rates of absorption for compounds of the benzothiazole class. It is not possible, therefore, to predict the toxic hazard posed by these substances when administered by various routes, and this is suggestive of the need for further research.

It is well-known that the relationship between toxicity and mode of absorption for any foreign compound is largely dependent upon its molecular structure, lipid solubility, degree of ionization, and vapor pressure. Unfortunately, data are not available to indicate the extent of ionization of MBT and its derivatives at the various pH values along the gastrointestinal tract. Furthermore, the fact that most MBT derivatives are readily organosoluble (i.e., lipid soluble) is not sufficient to predict their ease of absorption by oral and dermal routes.

With respect to the benzothiazoles used in industrial solutions, however, the route leading to the greatest absorption is most likely to be passage of inhaled dust through the alveoli of the lungs. In contrast to the skin and the gastrointestinal lining, the pulmonary epithelium appears to be highly permeable not only to lipid-soluble molecules but also to large lipid-insoluble molecules and ions (Enna and Schanker, 1959).

The processes of distribution and storage subsequent to absorption of a benzothiazole compound are largely unknown. In one study (Lehman, 1965) rats were fed a mixture of MBT (2.7%) and dimethyldithiocarbamate (27.6%) at 10,000 ppm in the diet for one week. A bacteriostatic assay method sensitive to 50 ug/g of tissue was employed to detect concentrations of these compounds in various body tissues. Storage of MBT could not be demonstrated in the liver, kidney or spleen. This study did not attempt to define, however, the extent of storage in the fat, which is an important consideration with lipid-soluble molecules such as MBT.

b. Metabolism Studies

The biotransformation of MBT in the rat has been investigated by Colucci and Buyske (1965). Adult male rats were administered 50 mg/kg of 2-^{35}S-MBT by intraperitoneal injection and the urine from these animals collected quantitatively for up to two weeks. An analysis of urinary metabolites revealed: (1) the presence of MBT with the same specific radioactivity as the dosed compound, (2) benzothiazole-2-mercaptoglucuronide with the same specific radioactivity as the dosed compound, (3) benzothiazole-2-mercaptoglucuronide with a slightly lower specific radioactivity than the parent compound, and (4) non-radioactive benzothiazole-2-mercapturic acid. Radioactive inorganic sulfate was also found in the urine.

The excretion of a non-radioactive metabolite of MBT immediately suggested the existence of a mechanism whereby the radioactive mercapto sulfur is detached from MBT, an idea which was supported by the presence of radiolabelled inorganic sulfur in the urine. The authors postulated a mechanism whereby glutathione or cysteine acted as a displacing agent which attached to the benzothiazole structure, a reaction which would lead to the formation of a mercapturic acid conjugate.

The presence in urine of benzothiazole-2-mercaptoglucuronide with lower specific activity than the parent compound was consistent with the authors' theory that a dilution of radioactive MBT with non-radioactive MBT would occur by the following reaction series:

$$R\text{-}^{35}SH \longrightarrow R\text{-}S\text{-glutathione} \longrightarrow R\text{-}SH \longrightarrow R\text{-}S\text{-glucuronide}$$

An overall diagrammatic representation of the metabolic fate of MBT is presented in Figure 7. It should be noted that when the same *in vivo* studies were performed in the rabbit and dog, the results were similar to those found in the rat study (Colucci and Buyske, 1965).

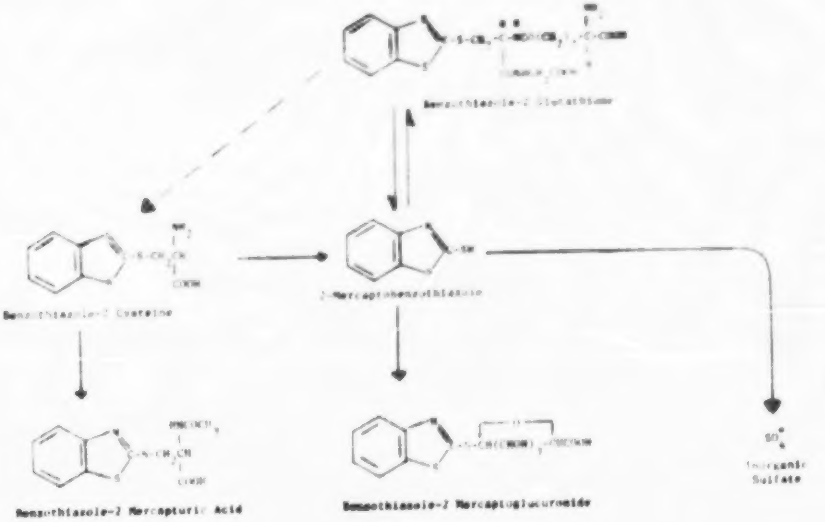

Benzothiazole-2 Glutathione
Benzothiazole-2 Cysteine
2-Mercaptobenzothiazole
$SO_4^=$
Inorganic Sulfate
Benzothiazole-2 Mercapturic Acid
Benzothiazole-2 Mercaptoglucuronide

Figure 7. Pathway for the Metabolic Transformation of 2-Mercaptobenzothiazole in the Rat, Rabbit, and Dog (data from Colucci and Buyske, 1965)

Colucci and Buyske (1965) were able to show further that 2-benzothiazolesulfonamide was extensively metabolized in the rat, rabbit, and dog by the same mechanism as for MBT. When 2-^{35}S-labelled 2-benzothiazolesulfonamide (100 mg/kg) was administered to the rat by intraperitoneal injection, three urinary metabolites were isolated and identified: (1) MBT, (2) benzothiazole-2-mercapturic acid, and (3) benzothiazole-2-mercaptoglucuronide. Surprisingly, none of the metabolites were radioactive; all of the radioactivity in the urine was due to radioactive inorganic sulfate. Similar results were also obtained in the rabbit and dog. From this data, it was apparent that the sulfonamide group was completely cleaved from the benzothiazole moiety. As with MBT, the thiol group of glutathione or cysteine could be the origin of the sulfur atom at the 2-position of the benzothiazole-containing metabolites recovered in the urine. This conclusion was supported by investigating the metabolism in the rat of 2-^{35}S-benzothiazole-2-glutathione. Isolation of urinary metabolites revealed that the same metabolites were produced as when 2-benzothiazolesulfonamide was given, except that all three metabolites contained radioactive sulfur at the 2-position. Furthermore, administration of benzothiazole-2-cysteine led to the formation of benzothiazole-2-mercapturic acid, benzothiazole-2-mercaptoglucuronide, and MBT as a minor metabolite. In vitro studies confirmed that the rat is capable of enzymatically cleaving the sulfur-carbon bond of this conjugate to form MBT. Therefore, the metabolic scheme as pictured in Figure 7 also applies to the sulfonamide derivative of benzothiazole.

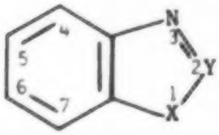
If one extrapolates these results to other benzothiazole derivatives having a sulfur linkage at the 2-position, it is possible that many of the commonly used rubber accelerators may also be metabolized in the manner portrayed by Figure 7. Considering the data which have been presented, it is not likely that MBT-type compounds will be metabolically transformed into substances of potentially high toxicity. These results should not be directly extrapolated, however, to benzothiazole derivatives not having a 2-position sulfur linkage; especially since S-glucuronide formation, although it occurs with MBT, is a relatively uncommon metabolic reaction (Miettinen and Leskinen, 1970).

c. Pharmacology

Benzothiazole and its various substituted derivatives possess distinct pharmacologic properties which are somewhat varied and highly structure-specific. Many of these substances were synthesized as potential chemotherapeutic agents for various uses such as antimicrobial drugs, anti-inflammatory agents, and diuretics. An examination of the data obtained in screening numerous benzothiazole derivatives for pharmacologic activity reveals a broad spectrum of biological activity, concerned mainly with actions on the central nervous system and effects on enzyme function.

Neurologic Effects

A detailed investigation of the pharmacologic properties of several benzothiazoles was undertaken by Domino *et al*. (1952) after it was revealed that the benzimidazoles, a structurally-related class, could produce a reversible flaccid paralysis in various animal species. Their examination of the benzothiazoles began with a determination of the paralyzing potency of several benzothiazoles upon their intravenous administration to mice. Median paralyzing doses (PD_{50}) and standard errors were calculated for these and several other chemicals described by the generic term of benzazoles (Figure 8).



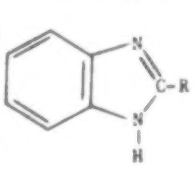
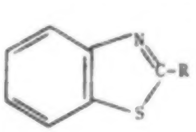
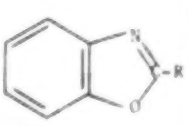
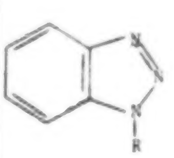
Benzimidazole X = NH Y = CH
Benzothiazole X = S, Y = CH
Benzoxazole X = O, Y = CH
Benzotriazole X = NH Y = N

Figure 8. Structural Formula of a Benzazole (from Domino *et al*., 1952)

A comparison is made in Table 21 of the relative paralyzing activities of these compounds.

Table 21. Median Paralyzing Doses of Substituted Benzazoles on Intravenous Administration to White Mice (Domino *et al*., 1952)

All doses are given in mgm/kgm ± standard error. Values without standard errors are approximate.

R	BENZIMIDAZOLES 	BENZOTHIAZOLES 	BENZOXAZOLES 	BENZOTRIAZOLES 
	PD_{50}	PD_{50}	PD_{50}	PD_{50}
H	86 ± 12	68 ± 8	167 ± 20	55 ± 3
CH_3	50	74 ± 7	-	230
CH_2CH_3	50	-	-	50
$\underline{n}C_5H_{11}$	Convulsions	-	-	
NH_2	Convulsions	20 ± 1	54 ± 2	
$NHCH_2$	-	25 ± 2		
$N(CH_3)_2$	75	81 ± 2		

Administration of benzothiazole was reported to cause an immediate increase in animal activity, such as running, hopping, and squeaking, which was followed in a few seconds by progressive ataxia leading to a loss of the righting reflex. A semi-flaccid paralysis ensued which began in the hind limbs and was reversible at non-lethal doses. In addition, respiratory depression was produced at paralyzing doses, a phenomenon which was also caused by benzoxazole but not benzimidazole or benzotriazole. 2-Methylbenzothiazole also caused initial running movements and respiratory depression during the paralysis period. The 2-hydroxy and 2-phenyl derivatives of benzothiazole were administered orally, due to their insolubility, and produced paralysis at doses of 1,000 and 1,500 mg/kg of body weight, respectively.

The 2-amino substitution of benzothiazole produced a compound with the greatest paralyzing potency of any substance tested. A stepwise replacement of the amino group hydrogens by methyl groups caused a progressive reduction in paralyzing activity, but these derivatives were capable of producing respiratory depression, as well as clonic convulsions.

Overall, it appears that the benzothiazoles are capable of causing selective actions on the central nervous system which may result in either stimulation or depression. These effects vary considerably depending on the species of animal, the route of administration, and the stereo-specific addition of substituents to the molecule. For example, most of the substituted 2-aminobenzothiazoles were depressants in mice and rabbits, while in dogs the 4-position derivatives were stimulants and the 7-position derivative was a convulsant. Domino and coworkers (1952) noted that the pharmacologic effects of these compounds were related to structure, such that methyl or chloro groups substituted in the benzene ring of the 2-aminobenzothiazole produced depression according to the following order: 6>5>4>7. 6-Methyl-2-aminobenzothiazole was found to be remarkably free of any stimulating effects, whereas the 7-substituted derivatives could produce a high degree of stimulation.

Effects on Enzymes In Vivo

The ability of MBT to act as a chelating agent has been linked to the observation that MBT causes an inhibition of the oxidative conversion of dopamine to noradrenaline. Noradrenaline (norepinephrine, levartarinol) is a neurohumoral transmitter released from sympathetic postganglionic nerves (adrenergic nerves). Norepinephrine is synthesized in adrenergic nerves from the amino acid tyrosine by the following sequence:

TYROSINE
↓ tyrosine hydroxylase
DOPA
(dihydroxyphenylalanine)
↓ dopa decarboxylase
DOPAMINE
(dihydroxyphenylamine)
↓ dopamine β-hydroxylase
NORADRENALIN

Adrenergic nerves supply stimuli primarily to the smooth muscle of the heart, blood vessels, lungs, gastrointestinal tract, urinary bladder, and other organs.

In addition, adrenergic receptors are located on arterioles in skeletal muscle.

Johnson _et_ _al_. (1970) recognized that the role of cupric ions was critical to the activity of dopamine β-hydroxylase, an enzyme which catalyzes the conversion of dopamine to noradrenaline. This enzyme, therefore, would be subject to inactivation by copper chelating agents, one of which is MBT. Johnson and his coworkers (1970) administered MBT at 300 mg/kg by intraperitoneal injection to mice and measured its effect on catecholamine levels in the brain. As indicated in Table 22, MBT reduced noradrenaline to about 60% of control levels when measured after 1 and 2 hours. Dopamine levels, on the other hand, were elevated by 24% at 2 hours. After 4 hours, both noradrenaline and dopamine levels had returned to normal.

Table 22. Mouse Brain Catecholamine Levels 1, 2, and 4 h After 2-Mercaptobenzothiazole (MBT), 300 mg/kg, i.p. (from Johnson _et_ _al_., 1970)

All values are expressed as μg/g wet weight whole brain tissue and are the average of at least three determinations ± s.e.

		Noradrenaline	Dopamine
Diluent treated controls		0.43 ± 0.03	0.78 ± 0.06
MBT	1 h	0.25 ± 0.01*	0.86 ± 0.02
	2 h	0.27 ± 0.02*	0.96 ± 0.03**
	4 h	0.42 ± 0.00	0.81 ± 0.02

* = P<0.01
** = P<0.05

The MBT treatment quickly produced signs of extreme depression in the mice, which was accompanied by marked ptosis (drooping of the eyelids) after 2 hours. Symptoms of overt depression subsided as catecholamine levels returned to normal.

A further study was made in rats to determine the effects of MBT on the repletion of myocardial noradrenaline from exogenous dopamine after the depletion of noradrenaline stores by injection of metaraminol bitartrate. Rats were treated with metaraminol (5 mg/kg) by intraperitoneal injection to deplete noradrenaline stores in the heart, and followed 18 hours later by intraperitoneal injection with 300 mg/kg of MBT. Thirty minutes after MBT treatment, dopamine was administered at 35 mg/kg, and three hours later the rats were sacrificed. As indicated in Table 23, exogenous dopamine restored myocardial noradrenaline concentrations to 60% of the control level. MBT, however, totally blocked the conversion of endogenous and exogenous dopamine to newly synthesized noradrenaline.

Table 23. Effect of 2-Mercaptobenzothiazole (MBT) on the Repletion of Rat Myocardial Noradrenaline from Exogenous Dopamine After Its Depletion with Metaraminol (from Johnson _et al_., 1970)

Rats were pretreated with metaraminol bitartrate, 5 mg/kg, 18 h before each received MBT, 300 mg/kg, or diluent. Dopamine hydrochloride, 35 mg/kg, or diluent was administered 30 min later and all rats were killed 3 h later. All values are expressed as μg/g and are the average of at least three determinations ± s.e.

	Myocardial noradrenaline
Diluent treated controls	0.88 ± 0.05
Metaraminol	0.15 ± 0.01
Metaraminol + dopamine	0.52 ± 0.09*
Metaraminol + MBT	0.18 ± 0.03
Metaraminol + MBT + dopamine	0.17 ± 0.02

*Significantly different from each of the other values, $P<0.05$.

When Johnson and his coworkers measured the effect of MBT on spontaneous motor activity in mice (Figure 9), their results coincided with the previously observed time-course of depletion of brain noradrenaline stores. Treated mice displayed almost no exploratory activity from the time they received MBT, as determined in an actophotometer cage. Spontaneous activity increased in treated mice after two hours, and coincided with the time of termination of dopamine β-hydroxylase inhibition in the brain.

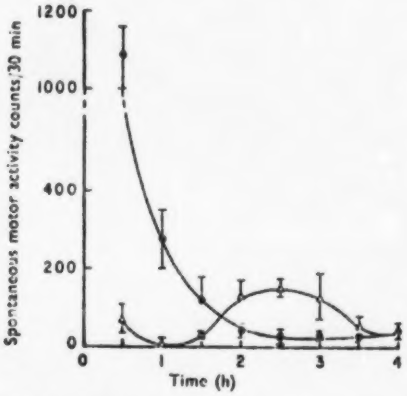


Figure 9. Effect of 2-Mercaptobenzothiazole, 300 mg/kg i.p., Upon Spontaneous Motor Activity in Mice. Each point represents the average 30 min. activity for six pairs of mice ± s.c. ——○——, control; ——△——, MBT. (from Johnson _et_ _al_., 1970)
(Reprinted with permission from the Pharmaceutical Society of Great Britain)

Numerous derivatives of benzothiazole were tested by Wattenberg _et_ _al_. (1968) for their ability to induce the increased activity of benzopyrene hydroxylase, an enzyme which has the capacity to metabolize foreign compounds. The liver is thought to be highly responsive to inducers and is generally used as a standard tissue for studying induction of increased activity in microsomal enzyme systems. Wattenburg and coworkers (1968) measured benzopyrene hydroxylase activity in the liver and also in the lung after treating rats by oral intubation with 0.1 mmole of one of 33 benzothiazole derivatives (Table 24). A comparison of enzyme activity in the liver and lung offers an indication of the possibility for selective induction in specific tissues other than the liver.

The data presented in Table 24 clearly indicate that MBT is not an inducer of increased benzopyrene hydroxylase activity in either the liver or lung. On the other hand, benzothiazole and several substituted derivatives produced significant inducing effects. The greatest degree of enzyme activity resulted from treatment with a 2-phenylbenzothiazole, especially those with certain halogen substitutions (i.e., chloro, bromo, and iodo, but not fluoro) in the 4'-phenyl position. As pointed out by the authors, the significance of any compound which markedly increases liver microsomal enzyme activity is that a potentially high number of undesirable side reactions may also be induced in the same organ. An inducer with specific activity on tissues other than liver (e.g., lung, gastrointestinal tract), however, would be capable of increasing detoxification activity in that organ without producing complications which might occur from elevated liver microsomal activity.

d. Acute Toxicity

Several animal studies have been conducted on the single-dose toxicity of numerous benzothiazole derivatives. Simple molecular modifications of benzothiazole produce large differences in toxic potency. For this reason, data on non-commercial benzothiazole derivatives are included in this section for the sake of relating specific structures and substitutions to their resultant toxic effects. Consequently, comparisons can readily be made, not

Table 24. Effects of 2-Phenylbenzothiazoles and Related Compounds on Benzopyrene Hydroxylase Activity of Rat Liver and Lung (from Wattenberg *et al*., 1968)

Compound tested[a]	Benzopyrene hydroxylase activity			
	Liver (units/mg wet weight[b])	Lung (units/mg wet weight[b])	(Ratio: Liver test/control[c])	(Ratio: Lung test/control[c])
Controls	13 ± 4	0.88 ± 0.24		
2-Phenylbenzothiazole	46 ± 8	3.40 ± 0.61	3.5	3.9
2-(2'-Bromophenyl)-benzothiazole	54 ± 7	4.42 ± 0.54	4.1	5.0
2-(3'-Bromophenyl)-benzothiazole	48 ± 5	2.07 ± 0.31	3.5	2.4
2-(4'-Bromophenyl)-benzothiazole	104 ± 3	6.46 ± 0.34	8.0	7.3
2-(2'-Chlorophenyl)-benzothiazole	38 ± 4	3.74 ± 0.85	2.9	4.2
2-(3'-Chlorophenyl)-benzothiazole	32 ± 5	1.70 ± 0.27	2.5	1.9
2-(4'-Chlorophenyl)-benzothiazole	100 ± 8	5.65 ± 1.53	7.8	6.4
2-(2',4'-Dichlorophenyl)-benzothiazole	42 ± 8	3.98 ± 0.58	3.2	4.5
2-(4'-Iodophenyl)-benzothiazole	98 ± 4	4.08 ± 0.71	7.5	4.6
2-(4'-Fluorophenyl)-benzothiazole	33 ± 7	2.38 ± 0.43	2.5	2.7
2-(4'-Aminophenyl)-benzothiazole	54 ± 3	3.64 ± 0.98	4.2	4.1
2-(4'-Hydroxyphenyl)-benzothiazole	19 ± 1	1.04 ± 0.27	1.5	1.2
2-(4'-Methoxyphenyl)-benzothiazole	27 ± 4	2.38 ± 0.22	2.1	2.7
2-(4'-Carboxyphenyl)-benzothiazole	16 ± 1	0.85 ± 0.17	1.2	1.0
2-(4'-Cyanophenyl)-benzothiazole	27 ± 1	4.08 ± 0.41	2.1	4.6
2-(4'-Methylphenyl)-benzothiazole	26 ± 1	1.57 ± 0.31	2.0	1.8
2-(4'-Formylphenyl)-benzothiazole	15 ± 1	0.75 ± 0.24	1.2	0.9
6-Methyl-2-phenylbenzothiazole	31 ± 5	3.06 ± 0.48	2.4	3.5
5-Chloro-2-phenylbenzothiazole	50 ± 7	3.74 ± 0.48	3.8	4.2
Benzothiazole	26 ± 4	1.36 ± 0.24	2.0	1.5
2-Mercaptobenzothiazole	14 ± 1	0.92 ± 0.17	1.1	1.0
2,2'-Bibenzothiazole	22 ± 1	0.85 ± 0.17	1.7	1.0
2,2'-Thiobisbenzothiazole	31 ± 0	0.92 ± 0.17	2.4	1.0
2,2'-Dithiobisbenzothiazole	19 ± 3	0.78 ± 0.17	1.5	0.9
2-Benzylthiobenzothiazole	34 ± 1	1.70 ± 0.27	2.6	1.9
2-Benzoylthiobenzothiazole	10 ± 0	0.78 ± 0.17	0.8	0.9
2-Phenoxybenzothiazole	18 ± 0	1.26 ± 0.41	1.4	1.4
2-Benzyloxybenzothiazole	33 ± 11	2.11 ± 0.44	2.5	2.4
2-Benzamidobenzothiazole	31 ± 3	2.38 ± 0.37	2.4	2.7
2-Benzylaminobenzothiazole	19 ± 1	1.36 ± 0.31	1.5	1.5
2-p-Toluenesulfonamidobenzothiazole	16 ± 0	0.92 ± 0.17	1.2	1.0
2-(4'-Pyridyl)-benzothiazole	33 ± 5	2.14 ± 0.53	2.5	2.4
2-Phenylbenzimidazole	15 ± 2	1.40 ± 0.24	1.2	1.6

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[a] 0.1 mmole of each compound in 1 ml dimethylsulfoxide was administered by stomach tube to 48-day-old female Sprague-Dawley rats 48 hours prior to sacrifice. Controls received vehicle only. 3-6 rats per group except for 2-phenylbenzothiazole, 15 animals and controls, 33 animals.

[b] Mean ± S.D.

[c] Ratio of benzopyrene hydroxylase activity of tissue from animal receiving the indicated compound divided by the activity of the control.

only among important commercial compounds but also between these substances and structurally-related derivatives. The available evidence indicates that acute intoxication by any of the benzothiazole derivatives will be manifested primarily as disruption of central nervous system function.

Oral and Parenteral Routes

A particularly detailed investigation of the toxicity of MBT was undertaken by Guess and O'Leary (1969) to help assess the potential public health hazards of rubber-containing items. The authors included in their study the compound 2-(2-hydroxyethylmercapto)benzothiazole (HMBT), a substance formed from MBT contained in rubber by reaction with ethylene oxide under conditions used to sterilize medical grade rubber articles.

Single-dose toxicity and LD_{50} determinations were made in mice, both by the oral and intraperitoneal routes. White male mice, 18 to 22 grams in weight, were administered either MBT or HMBT suspended in cottonseed oil for intraperitoneal injection, or in 0.5% carboxymethyl cellulose in 0.9% saline solution for the oral dosing. The results of the LD_{50} evaluations are presented in Table 25.

Deaths, when they occurred, were always within 24 hours. The signs of toxicity were more dramatic in those animals receiving higher doses of MBT than in those receiving HMBT by intraperitoneal injection. However, a comparison of the LD_{50}'s and maximum tolerated doses by either the oral or parenteral route indicated that HMBT may be more toxic than MBT. Nevertheless, for both compounds, doses in excess of 335 mg/kg induced a marked peripheral vasodilation, extensive salivation, and convulsions. Convulsions produced by MBT

Table 25. LD_{50} Values for MBT and HMBT in Mice (from Guess and O'Leary, 1969)

Route and statistic	MBT	HMBT
Intraperitoneal		
LD50	437 mg/kg	417 mg/kg
Approx. 95% C.L.[a]	415-461 mg/kg	390-455 mg/kg
Probit slope	19.05 probits/log	12.38 probits/log
Max. tolerated dose	305 mg/kg	239 mg/kg
Oral		
LD50	2000 mg/kg	1017 mg/kg
Approx. 95% C.L.	1798-2225 mg/kg	885-1146 mg/kg
Probit slope	9.01 probits/log	6.59 probits/log
Max. tolerated dose	931 mg/kg	357 mg/kg

[a] Confidence limit

became progressively severe, with intermittent clonic and tonic seizures of prolonged duration. HMBT, on the other hand, induced convulsions of the spasmodic "leaping" type accompanied by long periods of quiet inactivity.

The overt symptoms of intoxication by MBT and HMBT were clearly suggestive of central nervous system action. Other benzothiazole derivatives are well-known for their central nervous effects (see Section III-B-2-c, p. 79). Additional studies were conducted to determine whether the salivation and slight lacrimation seen at higher dose levels were due to cholinergic activity mediated through the autonomic system. As a test, atropine sulfate (0.2 mg/kg) was given intraperitoneally 30 minutes prior to dosing with 550 mg/kg MBT or HMBT. This treatment did not block the salivation activity and only slightly reduced peripheral vasodilation. Therefore, cholinergic activity was discounted as the mode of toxic action, and instead the possibility of central stimulation via the cervical sympathetic ganglion was considered. Predictably, the intraperitoneal injection of pentobarbital (25 mg/kg) 30 minutes prior to treatment with 550 mg/kg MBT or HMBT completely blocked the convulsions and salivation. These results confirmed the role of the central nervous system as a major site of toxic action for MBT or HMBT. The peripheral vasodilation, however, which was induced by MBT or HMBT treatment could be markedly reduced, but the effect persisted nevertheless.

The acute oral and parenteral toxicities of several benzothiazole derivatives used as rubber accelerators have been investigated along with those of many non-commercial substituted benzothiazoles. Table 26 summarizes these results. An examination of the data contained in the table reveals that substituents of increasing size added to the 2-position of benzothiazole tend to decrease acute toxicity. Unsubstituted benzothiazole and several benzene ring-substituted 2-aminobenzothiazoles appear to be the most toxic derivatives of the class. It is not known to what extent the parameters of absorption, tissue and plasma binding, and drug-gastrointestinal tract interactions may influence the toxicity of individual compounds. In general, it has been shown that the mouse is more susceptible than are the rat and guinea pig; sufficient data are not available to evaluate the modifying effects of factors such as sex or strain differences.

Table 26. Acute Animal Toxicity of Benzothiazole Derivatives

Substance	Species	Sex & (No.)	Dose (mg/kg)	Route of Administration	Effects	Reference
2-Aminobenzothiazole	Mouse	M (?)	200 adjusted to pH = 7.0 with H_2O + NaOH	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Mouse	?	126	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-4-chlorobenzothiazole	Mouse	?	2400	oral	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
	Mouse	?	71	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-5-chlorobenzothiazole	Mouse	?	92	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-6-chlorobenzothiazole	Mouse	?	398	oral	LD_{50}, temporary flaccid paralysis in survivors	Domino et al., 1952
	Mouse	?	76	i.v.	LD_{50}, temporary flaccid paralysis in survivors	Domino et al., 1952
2-Amino-7-chlorobenzothiazole	Mouse	?	77	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-4,6-dimethylbenzothiazole	Mouse	?	850	oral	LD_{50}, paralysis lasting 12 to 18 hours with death ensuing by progressive respiratory depression	Domino et al., 1952
2-Amino-4-ethoxybenzothiazole	Mouse	?	80	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-4-ethylbenzothiazole	Mouse	?	77	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-4-hydroxybenzothiazole	Mouse	?	160	i.v.	LD_{50}, temporary paralysis and marked hyperpnea in survivors	Domino et al., 1952

KEY

[a] CMC = carboxymethyl cellulose; PG = propylene glycol

[b] i.p. = intraperitoneal; i.v. = intravenous

[c] LD_{50} = calculated dose causing death in 50% of the experimental animal species

[d] dosages calculated from milliliter equivalents of a 50% solution

Table 26. (cont'd)

Substance	Species	Sex & (No.)	Dose (mg/kg)	Route of Administration	Effects	Reference
2-Amino-6-hydroxybenzothiazole	Mouse	?	300	i.v.	LD_{50}, clonic convulsions	Domino et al., 1952
6-Amino-2-mercaptobenzothiazole	Mouse	M (?)	150-200 adjusted to pH = 7.0 with H_2O + NaOH	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Mouse	?	>300 in CMC	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
2-Amino-4-methoxybenzothiazole	Mouse	?	562	oral	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
	Mouse	?	46	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-5-methoxybenzothiazole	Mouse	?	150	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-6-methoxybenzothiazole	Mouse	?	140	i.v.	LD_{50}, running convulsions and respiratory depression	Domino et al., 1952
2-Amino-4-methylbenzothiazole	Mouse	?	697	oral	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
	Mouse	?	54	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-5-methylbenzothiazole	Mouse	?	1070	oral	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
	Mouse	?	74	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
2-Amino-6-methylbenzothiazole	Mouse	M (?)	>100 in CMC	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Mouse	?	525	oral	LD_{50}, temporary flaccid paralysis in survivors	Domino et al., 1952
	Mouse	?	84	i.v.	LD_{50}, temporary flaccid paralysis in survivors	Domino et al., 1952

KEY

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[c] LD_{50} = calculated dose causing death in 50% of the experimental animal species

[d] dosages calculated from milliliter equivalents of a 50% solution

Table 26. (cont'd)

Substance	Species	Sex & (No.)	Dose (mg/kg)	Route of Administration	Effects	Reference
5-Amino-2-phenylbenzothiazole	Rat	?	2940	oral	LD_{50}	Christensen and Luginbyhl, 1974
Benzothiazole	Mouse	M (?)	100-200 in PG + H_2O	i.p.	Approximate LD_{50} after 7 days	Doull *et al.*, 1962
	Mouse	?	95	i.v.	LD_{50}, temporary paralysis in survivors	Domino *et al.*, 1952
	Mouse	?	100[d]	i.v.	LD_{50}	Christensen and Luginbyhl, 1974
N-*tert*-Butyl-2-benzothiazolesulfenamide	Rat	M (10)	10,000	oral	No mortality; animals became sluggish within 15 minutes and displayed piloerection within 45 minutes; nothing remarkable on autopsy	American Cyanamid, 1975
2-Chlorobenzothiazole	Mouse	M (?)	200-300 in PG + H_2O	i.p.	Approximate LD_{50} after 7 days	Doull *et al.*, 1962
5-Chloro-2-methylbenzothiazole	Mouse	M (?)	500 in PG	i.p.	Approximate LD_{50} after 7 days	Doull *et al.*, 1962
N-Cyclohexyl-2-benzothiazolesulfenamide	Mouse	M (?)	>2500 in CMC	i.p.	Approximate LD_{50} after 7 days	Doull *et al.*, 1962
	Mouse	?	>4000 in oil	oral	LD_{50}	Vorob'eva and Mezentseva, 1962
	Rabbit	?	4000	oral	LD_{50}	Christensen and Luginbyhl, 1974
	Mouse	?	1870	oral	LD_{50}	Christensen and Lubinbyhl, 1974
	Rat	M (10)	10,000	oral	One death. In victim, liver and spleen mottled; kidneys mottled and congested; adrenals slightly congested; stomach distended, filled with a hard mass; intestines liquid-filled, yellow and hemorrhaged. Nothing remarkable in survivors. No mortality produced at a dosage of 5000 mg/kg (3 rats).	American Cyanamid, 1975

KEY

[a] CMC = carboxymethyl cellulose; PG = propylene glycol

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[c] LD_{50} = calculated dose causing death in 50% of the experimental animal species

[d] dosages calculated from milliliter equivalents of a 50% solution

Table 26. (cont'd)

Substance	Species	Sex & (No.)	Dose (mg/kg)	Route of Administration	Effects	Reference
2,6-Diaminobenzothiazole	Mouse	M (?)	>1000 in PG	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Mouse	?	384	i.v.	LD_{50}, running convulsions and respiratory depression	Domino et al., 1952
N,N-Dicyclohexylbenzothiazole-sulfenamide	Rat	?	3450	oral	Lethal dose	Christensen and Luginbyhl, 1974
2-(Dimethylamino)-benzothiazole	Mouse	?	131	i.v.	LD_{50}, temporary paralysis in survivors	Domino et al., 1952
N,N-Diisopropyl-2-benzothiazolesulfenamide	Rat	M (10)	10,000	oral	Three deaths (1 on day 3; 2 on day 4). In victims, livers and spleens mottled; kidneys speckled and slightly congested; intestines liquid-filled and hemorrhaged. Nothing remarkable in survivors. No overt signs of intoxication were noted.	American Cyanamid, 1975
	Rat	M (3)	5000	oral	One death on day 2. In victim, results of autopsy same as above. No overt signs of intoxication were noted.	American Cyanamid, 1975
	Mouse	M (?)	3892	?	LD_{50}	Vorob'eva, 1968
2,5-Dimethylbenzothiazole	Rat	?	957	oral	LD_{50}	Christensen and Luginbyhl, 1974
2,2'-Dithiobisbenzothiazole	Mouse	M (?)	>2000	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Guinea Pig	?	2250	i.p.	LD_{50}; pathological changes in internal organs	Kowalski and Bassendowska, 1965
	Rat	?	>5000	oral	LD_{50}	R. T. Vanderbilt, 1975a

KEY

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[c] LD_{50} = calculated dose causing death in 50% of the experimental animal species

[d] dosages calculated from milliliter equivalents of a 50% solution

Table 26. (cont'd)

Substance	Species	Sex & (No.)	Dose (mg/kg)	Route of Administration	Effects	Reference
2-Hydrazinobenzothiazole	Mouse	M (?)	100-200 in PG	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
2-(2-Hydroxyethylmercapto) benzothiazole	Mouse	M (?)	1017	oral	LD_{50}; deaths within 24 hours; peripheral vasodilation, extensive salivation, convulsions	Guess and O'Leary, 1969
	Mouse	M (?)	417	i.p.	LD_{50}; death within 24 hours; symptoms same as above	Guess and O'Leary, 1969
MBTS Pellets (mixture containing: 76-80%, 2,2'-dithiobisbenzothiazole; 6-9%, 2-mercaptobenzothiazole; 5-7%, 2-benzothiazolyl-2-aminophenyl disulfide and 2,2'-diaminodiphenyl disulfide combined)	Rat	M (5)	10,000	oral	100% mortality within 24 hours; only sign of toxicity was depression; autopsy normal	American Cyanamid, 1975
	Rat	M (5) (5) (5)	5000, 2500, 1250	oral	All animals survived a 7-day observation period.	American Cyanamid, 1975
2-Mercaptobenzothiazole	Rat	?	3000	oral	LD_{50}	R.T. Vanderbilt, 1975b
	Mouse	?	2306	oral	LD_{50}	Vorob'eva and Mezentseva, 1968
	Mouse	?	1851	oral	LD_{50}	R.T. Vanderbilt, 1975b
	Mouse	M (?)	2000	oral	LD_{50}; deaths within 24 hours; peripheral vasodilation, extensive salivation, convulsions; intermittent clonic and tonic seizures of prolonged duration	Guess and O'Leary, 1969
	Mouse	?	1800 in oil	oral	LD_{50}	Vorob'eva and Mezentseva, 1962
	Mouse	M (?)	437	i.p.	LD_{50}; deaths within 24 hours; symptoms same as above	Guess and O'Leary, 1969
	Mouse	M (?)	100-200 in PG	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Mouse	M (?)	>300 adjusted to pH = 7.0 with H_2O + NaOH	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Guinea Pig	?	1680	oral	LD_{50}; pathological changes of internal organs	Kowalski and Bassendowska, 1965
	Guinea Pig	?	[illegible]	i.p.	LD_{50}; pathological changes of internal	Kowalski and Bassendowska, 1965

KEY

[a] CMC = carboxymethyl cellulose; PG = propylene glycol

[b] i.p. = intraperitoneal; i.v. = intravenous

[c] LD_{50} = calculated dose causing death in 50% of the experimental animal species

[d] dosages calculated from milliliter equivalents of a 50% solution

Table 26. (cont'd)

Substance	Species	Sex & (No.)	Dose (mg/kg)	Route of Administration	Effects	Reference
2-Mercaptobenzothiazole, sodium salt	Rat	?	3968	oral	LD_{50}	R. T. Vanderbilt, 1975c
	Rat	M (5)	2500	oral	100% mortality; tremors, convulsions, and death within 3-5 minutes; severe depression and hematuria; hemorrhage of stomach	American Cyanamid, 1975
	Rat	M (5)	1250	oral	3 of 5 rats died with tremors, convulsions, and death occurring within 3-5 minutes; severe depression and hematuria; hemorrhage of stomach in decedents. Survivors recovered after 2 days and autopsy was normal.	American Cyanamid, 1975
	Rat	M (5)	625	oral	2 of 5 rats died; tremors, convulsions, and death within 3-5 minutes; severe depression and hematuria; hemorrhage of stomach in decedents only	American Cyanamid, 1975
	Rat	M (5)	312.5	oral	1 of 5 rats died on day 2; tremors, convulsions; severe depression and hematuria noted; hemorrhage of stomach in decedent only.	American Cyanamid, 1975
2-Mercaptobenzothiazole, zinc salt	Mouse	M (?)	200-300 in PG	i.p.	Approximate LD_{50} after 7 days	Doull *et al.*, 1962
	Rat	?	540	oral	LD_{50}	R. T. Vanderbilt, 1975d

KEY

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[a] CMC = carboxymethyl cellulose; PG = propylene glycol

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[c] LD_{50} = calculated dose causing death in 50% of the experimental animal species

[d] dosages calculated from milliliter equivalents of a 50% solution

Table 26. (cont'd)

Substance	Species	Sex & (No.)	Dose (mg/kg)	Route of Administration	Effects	Reference
2-Methylbenzothiazole	Mouse	M (?)	300-500 in PG + H_2O	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Mouse	?	105	i.v.	LD_{50}, temporary paralysis in survivors	Doull et al., 1962
2-Methyl-mercaptobenzothiazole	Mouse	M (?)	200-300 in PG + H_2O	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
4-Morpholinyl-2-benzothiazyl disulfide	Rat	?	>16,000	oral	LD_{50}	R. T. Vanderbilt, 1975e
6-Nitro-2-mercaptobenzothiazole	Mouse	M (?)	25-100 in CMC	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
N-Oxydiethylene-2-benzothiazolesulfenamide	Mouse	M (?)	100-200 in PG	i.p.	Approximate LD_{50} after 7 days	Doull et al., 1962
	Mouse	?	1980 in oil	oral	LD_{50}	Vorob'eva and Mezentseva, 1962
	Mouse	?	4000	oral	LD_{50}	R. T. Vanderbilt, 1975f
	Rat	M (10)	10,000	oral	One death. In victim, liver and spleen mottled; kidneys congested; stomach transparent; pylorus hemorrhaged; intestines pink, gas and chemical-filled. Animals became sluggish within 15 minutes, displayed pilo-erection within 30 minutes, and were prostrate within 35 minutes. Spleens were dark in survivors. No mortality produced at dosage of 5000 mg/kg (3 rats).	American Cyanamid, 1975

KEY

[a] CMC = carboxymethyl cellulose; PG = propylene glycol

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[d] dosages calculated from milliliter equivalents of a 50% solution

Skin and Eye Irritation

Guess and O'Leary (1969) looked further into the consequences of acute exposure to MBT and HMBT by determining their primary irritant activity and effect on wound healing. Suspensions of MBT and HMBT were prepared in cottonseed oil or carboxymethyl cellulose-saline solution at concentrations of 4%, 2%, 1% and 0.5%. Intradermal injections were then made at four sites each into the shaven backs of four albino rabbits at a dose of 0.2 ml per site. Only MBT at a 4% concentration in oil consistently induced intradermal irritation. This irritation, however, was mild and transitory, less than that shown by a 20% ethanol solution. HMBT in oil or aqueous suspension did not produce obvious skin irritation. Histologic examination of skin tissue after treatment with 4% MBT or HMBT demonstrated a mild inflammation at 24 hours with MBT, but no signs of irritation with either substance after 48 hours.

The effect of MBT and HMBT on wound healing was evaluated in rabbits bearing four superficial incisions on the back, 1 cm long, 2 mm deep, and 0.5 cm apart. Oil or aqueous suspensions of MBT or HMBT were applied to each area at concentrations of 4%, 2%, 1% and 0.5%, and the sites observed daily for evidence of erythema, scar formation, rate of healing, and other effects. In no instance was the normal healing process inhibited by either the MBT or HMBT treatment. Erythema and edema were absent, and hair growth and total healing time were identical for all rabbits.

Further tests on skin and eye irritation caused by a commercial mildew inhibitor containing MBT were conducted by the Department of the Army (Rowe, 1969). The compound tested was Vancide 51Z, a commercial fungicide containing as active ingredients 90% Ziram (zinc salt of dimethyl dithiocarbamic acid) and 7.8% zinc salt of MBT. Their results are summarized in Table 27.

Table 27. Primary Irritation Evaluation of Mildew Inhibitor Vancide 51Z (data from Rowe, 1969)

Test	Dose	Results
Skin Irritation - single 24-hour application to intact and abraded skin of rabbits	0.5 gram commercial grade powder applied to each of 4 rabbits	
	0.5 gram commercial grade powder in acetone (total volume 1.5 ml) applied to each of 4 rabbits	Vancide 51Z did not cause primary irritation of the intact skin or of the skin surrounding an abrasion when applied either as the commercial powder or the commercial grade formulation in acetone
Eye Irritation - single 24-hour application	0.1 gram commercial grade powder to one eye of each of 5 rabbits	Vancide 51Z caused destruction of all or nearly all of the corneal epithelium as evidenced by staining with Fluorescein. Also evident were copious ocular discharge and severe chemosis in every eye tested.
	0.2 ml of a 1% suspension of commercial grade powder in propylene glycol to one eye of each of 5 rabbits	Diluted Vancide 51Z caused slight corneal damage in all treated eyes. This formulation caused a moderate to heavy ocular discharge in every eye tested and moderate chemosis in 4 of 5 eyes tested.

Interpretation of these results by the author led to a recommendation calling for no restriction of acute application of Vancide 51Z to human skin. It was also recommended that the technical grade powder in undiluted form should be used with extreme caution and should be restricted to areas other than the face. In diluted form, it was suggested that Vancide 51Z be used with caution around the eyes and mucosa.

Eye irritation tests have also been conducted on several rubber accelerator chemicals belonging to the benzothiazole class (American Cyanamid, 1975). Groups of six rabbits were treated by instillation of the test substance directly to the eye, and the reactions evaluated at 24, 48, and 72 hours. Maximum possible scores for eye irritation (excluding necrosis) were: cornea, 80; iris, 10; conjunctivae, 20. The results, summarized in Table 28, indicate that sodium MBT is a particularly significant hazard to the eye. Corneal opacity was evident within 4 hours of dosing of the eyes and did not significantly improve during the following week.

Table 28. Acute Eye Irritation of Benzothiazole Derivatives in the Rabbit[a] (data from American Cyanamid, 1975)

Substance	Dose	Structure	Mean Value (24 hours)	Mean Value (48 hours)	Mean Value (72 hours)	Observations
Sodium mercaptobenzothiazole	50 mg.[b]	Cornea	30.0	38.3	38.3	Discernible opacity or ulceration of the cornea (other than a slight dulling of the normal luster); inflammation of the iris (other than slight deepening of the folds or slight circumcorneal injection); diffuse deep-crimson red appearance of the conjunctivae, with individual vessels not easily discernible; obvious swelling of the conjunctivae, excluding cornea and iris, with partial eversion of the lids; destruction or irreversible tissue change in 24 hours or less.
		Iris	3.3	4.1	4.1	
		Conjunctivae	13.0	13.0	13.0	
N-Oxydiethylene-2-benzothiazolesulfenamide	100 mg.	Cornea	15.0	11.7	10.0	Discernible opacity or ulceration of the cornea (other than a slight dulling of the normal luster); inflammation of the iris (other than slight deepening of the folds or slight circumcorneal injection); obvious swelling of the conjunctivae, excluding cornea and iris, with partial eversion of the lids.
		Iris	5.0	2.5	3.3	
		Conjunctivae	8.3	7.3	5.3	
N,N-Diisopropyl-2-benzothiazolesulfenamide	100 mg.	Cornea	2.5	0.0	0.0	Discernible opacity or ulceration of the cornea (other than a slight dulling of the normal luster).
		Iris	0.0	0.0	0.0	
		Conjunctivae	3.0	0.0	0.0	
N-Cyclohexyl-2-benzothiazolesulfenamide	100 mg.	Cornea	7.5	4.2	0.83	Discernible opacity or ulceration of the cornea (other than a slight dulling of the normal luster); inflammation of the iris other than slight deepening of the folds or slight circumcorneal injection.
		Iris	3.3	0.0	0.0	
		Conjunctivae	5.3	3.3	1.3	
N-*tert*-Butyl-2-benzothiazolesulfenamide	100 mg.	Cornea	10.0	6.7	4.2	Discernible opacity or ulceration of the cornea (other than a slight dulling of the normal luster).
		Iris	1.7	0.83	0.83	
		Conjunctivae	5.0	4.0	3.0	

[a] Compounds were instilled in one eye of each of six rabbits per treatment group, and reactions evaluated at 24, 48, and 72 hours. Maximum possible scores for irritation (excluding necrosis) were: cornea, 80; iris, 10; conjunctivae, 20.

[b] Dose was given as 0.1 ml of a 50% solution.

Dermal Toxicity

Tests to measure the toxic effects from dermal exposure to benzothiazole derivatives have only been conducted on several of the compounds used as rubber accelerators (Table 29). From the data presented, it is obvious that sodium MBT represents a significant hazard when applied to the skin. The apparent lack of toxicity for the other compounds tested is consistent with the low order of toxicity observed when these substances are administered orally (Table 26). In the absence of confirming data from parenteral or inhalation studies, it can only be suggested that the low toxic potency of the more complex benzothiazole accelerators may be a reflection of their poor absorption, or the fact that they are much less corrosive than sodium MBT.

e. Subacute Toxicity

Data from subacute studies should be interpreted with special consideration of the fact that target organs and responses of acute bioassays are often different than those following repeated exposure to sublethal doses. The phenomena of storage, metabolic activation, and repeated damage to organs and organelles are potential hazards of repeated exposure, and more closely resemble the consequences to man from environmental contamination. Massive single-dose exposures are generally of little practical use other than for estimating the potential to produce mortality.

Guess and O'Leary (1969) have shown that repeated exposure of mice to MBT at a dosage of one-fourth the LD_{50} can produce serious liver damage which is not detectable by gross observation. Male mice were treated daily for one week with intraperitoneal injections of MBT or HMBT [2-(2-hydroxyethylmercapto)benzothiazole] as either oil or aqueous suspensions. Groups were dosed at

Table 29. Acute Dermal Toxicity of Benzothiazole Derivatives in the Rabbit[a] (data from American Cyanimid, 1975)

Substance	Sex + (No.)	Dose (mg/kg)	Mortality died/dosed	Observations
Sodium mercaptobenzothiazole[b]	M (10) M (10) M (10)	2500 1250 625	4/10 1/10 0/10	Severe depression; cold extremities; appetite loss. Severe degree of skin injury; area burned at 24 hours with formation of hard eschar at 1-2 weeks. Gross autopsies were normal.
N-*tert*-Butyl-2-benzothiazolesulfenamide	M (5)	10,000	0/5	No signs of intoxication or skin irritation. Gross autopsies showed nothing remarkable.
N-Cyclohexyl-2-benzothiazolesulfenamide	M (5)	10,000	0/5	No signs of intoxication or skin irritation. Gross autopsies showed nothing remarkable.
N-Oxydiethylene-2-benzothiazolesulfenamide	M (5)	10,000	0/5	No signs of intoxication or skin irritation. Gross autopsies showed nothing remarkable.
N,N-Diisopropyl-2-benzothiazolesulfenamide	M (5)	10,000	0/5	No signs of intoxication of skin irritation. Gross autopsies showed nothing remarkable.
MBTS Pellets (mixture containing: 76-80%, 2,2'-dithiobisbenzothiazole; 6-9%, 2-mercaptobenzothiazole; 5-7%, 2-benzothiazolyl-2-aminophenyl disulfide and 2,2'-diaminodiphenyl disulfide (combined)	M (5)	10,000	0/5	No signs of toxicity during a 7-day observation period. Autopsies were normal. 3 of 5 animals lost weight.

[a] Compounds were applied to the clipped unabraded skin and held in contact by occlusive bandage for 24 hours.

[b] Dosages were calculated from milliliter equivalents of a 50% solution.

one-fourth and one-eighth the LD_{50} (110 and 55 mg/kg, respectively for MBT; 104 and 52 mg/kg, respectively for HMBT). At the dose levels for both compounds administered, no signs of overt toxicity could be detected after one week of injections. Weight gain and behavior patterns did not differ from control animals receiving the injection vehicle only. After sacrifice at the end of one week, gross examination of vital organs revealed no significant injury for either compound at all dose levels. Microscopic examination of various tissues, however, revealed that serious damage to the liver had occurred in those mice receiving MBT at one-fourth the LD_{50} dose. Results of the pathologic examination of all tissues are presented in Table 30. Tissues from mice receiving one-eighth the LD_{50} dose were not examined microscopically.

Table 30. Microscopic Pathologic Findings in Mouse Tissues After Dosing with MBT and HMBT for One Week (from Guess and O'Leary, 1969)

Compound	Liver	Lungs	Kidney	Heart	Thyroid	Testes
MBT	Necrosis	Normal	Cloudy swelling	Normal	Normal	Normal
HMBT	Cellular swelling	Normal	Normal	Normal	Normal	Normal

Sections of livers from MBT-dosed animals revealed extensive liver damage in the form of necrosis and reaction to the injury. At the cellular level, there was cloudy swelling, opacification, accumulation of various cytoplasmic granules, hyaline change, rupture of cell walls, and profound changes in nuclei. In addition, rupture of bile canaliculi and bile stasis had occurred which caused a marked inflammatory reaction with a severe infiltration of lymphocytes. The effects of HMBT on the anatomic organization of the liver were much less severe although some damage had occurred, primarily as cellular swelling and distinct cell walls.

In order to determine the effect of subacute treatment with MBT and HMBT on liver function, a hexobarbital narcosis study was conducted in mice. Aqueous saline suspensions of MBT and HMBT were given daily for one week by intraperitoneal injection at one-fourth or one-eighth the LD_{50} dose. After one week, hexobarbital sodium was given at 75 mg/kg and the resulting time of sleeping was judged as a measure of liver function (i.e., as liver function becomes impaired, sleep time will increase due to the decreased capacity of the organ to metabolize and eliminate the hexobarbital). As indicated in Table 31, both MBT and HMBT at the one-fourth LD_{50} dose caused a significant increase in sleeping time when compared to control animals. These results confirm the microscopic studies which showed that marked damage to the liver had occurred.

Table 31. Effect of MBT and HMBT on Hexobarbital Narcosis in Mice (from Guess and O'Leary, 1969)

Dose, ip	Number of mice	Sleep time ± SD (min)
MBT		
55 mg/kg	9	23.1 ± 4.94
110 mg/kg	8	25.0 ± 5.44[a]
HMBT		
52 mg/kg	8	21.0 ± 8.00
104 mg/kg	10	22.7 ± 2.54[a]
Control	8	17.0 ± 6.52

[a] Significantly different from control at P = 0.05

The results of a study have been reported which involved the subacute feeding of MBTS Pellets, a rubber accelerator product (American Cyanamid, 1975). MBTS Pellets is a mixture of 76-80% 2,2'-dithiobisbenzothiazole, 6-9% MBT, and 5-8% 2-benzothiazolyl-2-aminophenyl disulfide and 2,2'-diaminodiphenyl disulfide combined. This product was added to the diet of young male albino rats at concentrations of 0.5%, 1.0%, and 2.0% for a 31 day period. Table 32 shows that slight, but significant, reductions in mean daily food intake and mean total weight gain resulted in all treatment groups. There did not appear to be any dose-related relationship to these results.

Table 32. Summary of Results of 31 Daily Doses of MBTS Pellets in the Diet of Male Albino Rats (American Cyanamid, 1975)

Concentration in diet, %	0	0.5	1.0	2.0
Number of animals	10	10	10	10
Mean dosage, gm/kg/day	-	0.49	0.94	2.05
Mean food intake, gm/rat/day	18.5	17.5*	16.4*	17.8*
Mean weight gain, gm/rat	161	146*	141*	140*
Deaths	-	-	-	-
Mean no. of days to death	-	-	-	-

* Denotes a value significantly lower than control value ($p = .05$)

All animals were sacrificed and a gross examination performed for signs of pathology, but no remarkable effects could be found that might be due to the treatment.

A number of rubber accelerator chemicals related to MBT were tested for carcinogenicity and subacute toxicity in mice by Innes et al. (1969). Determinations were made of the maximum tolerated dose for each chemical. The maximal levels resulting in zero mortality were determined for a single oral dose, then for 6 daily doses, and finally for 19 daily doses (Table 33).

Table 33. Maximal Tolerated Doses of MBT and Derivatives in Mice (data from Innes et al., 1969)

Substance	Vehicle	Daily Dosage* (mg/kg)
2-Mercaptobenzothiazole	0.5% gelatin	100
2,2'-Dithiobisbenzothiazole	0.5% gelatin	464
Zinc salt of 2-mercaptobenzothiazole	0.5% gelatin	1000
N-Cyclohexyl-2-benzothiazolesulfenamide	0.5% gelatin	215
N-Oxydiethylene-2-benzothiazolesulfenamide	0.5% gelatin	464

* Given by stomach tube.

Further reports from the Russian literature (read in abstract) have described various effects due to MBT exposure. Litvinchuk (1963) reported that repeated doses of 50-200 mg/kg of MBT to rats (route unknown) caused no serious changes in respiration, blood picture, or renal functions. The treatment did, however, cause an increase in bile output (rats) and a 50% enhancement of gastric juice secretion in dogs. Vorob'eva et al. (1968) noted that extensive exposure to MBT dust (species unknown) affects respiration and the liver, but has no apparent effect on the nervous system. When Mikhailov (1973) exposed animals (species unknown) to daily oral doses of MBT at 2.5 to 25 mg/kg/day, he observed a decreased level of sulfhydryl groups in the blood serum.

Additional toxicity studies on several benzothiazole rubber accelerators have also been abstracted from the foreign literature. Vorob'eva and Mezentseva (1962, 1963) allowed rats to inhale dust of N,N-diisopropyl-2-benzothiazolesulfenamide at a concentration of 340-400 mg/m^3 for two hours daily on 15 days. No adverse changes were observed. When rabbits were treated orally with the same compound at 20 mg/kg for four months, disturbances were noted in the protein-forming function of the liver. Additional effects included elevation of blood serum alkaline phosphates and aldolase activity, but no behavioral changes or local irritant effects were noted.

Three sulfenamide rubber accelerators, N,N-diethyl-2-benzothiazolesulfenamide, N-oxydiethylene-2-benzothiazolesulfenamide, and N-cyclohexyl-2-benzothiazolesulfenamide (CBS) were evaluated in mice and rats by Zaeva et al. (1966). They demonstrated that rats were more sensitive to these compounds than mice, and that toxicity was greatest for CBS (minimum lethal oral dose = 5000 mg/kg), with N,N-diethyl-2-benzothiazolesulfenamide being the least toxic. All compounds inhibited thyroid function and caused dystrophic alterations in the liver and kidneys. CBS also caused a reduction of hemoglobin and erythrocytes in the blood. When CBS and N-oxydiethylene-2-benzothiazolesulfenamide were administered by intratracheal injection, they were found capable of causing pathological disturbances of the lungs (i.e., interstitial productive process, emphysema, and bronchitis). Inhalation of N,N-diethyl-2-benzothiazolesulfenamide at a concentration of 0.001 to 0.002 mg/l and repeated 25 times caused slight irritation and increased excitability of the nervous system. This compound also produced local irritation of the skin and mucous membranes.

f. Sensitization

It has previously been established that MBT is a common allergic contact sensitizer in humans (see Section III-B-1, p. 55). Presently, a number of animal models and screening methods are being employed for identification of contact allergens, and one of the most sensitive is the so-called "guinea-pig maximization test" (Magnusson and Kligman, 1969). This procedures involves a two-step induction phase whereby intradermal injections of the test substance, both with and without complete Freund's adjuvant, are given in the shoulder region. One week after the injections, the test agent is applied topically by closed patch to the same site. Challenge is made two weeks later by topical application with an occlusive patch to the flank region for 24 hours. The challenge site is evaluated 24 hours after removal of the patch. Allergenic potency is determined by percentage of animals sensitized, not intensity, and each substance tested is assigned to one of five grades ranging from 0, to weak (I), to extreme (V). Table 34 presents the results when MBT was assayed for allergenic capability by the "guinea-pig maximization test." By comparison, when MBT was tested by the conventional Landsteiner-Draize Test, the sensitization rate was zero. This result is not in accord with clinical experience as summarized in Section III-B-1, p. 55, and suggests that other derivatives of MBT should also be screened by maximization testing.

Table 34. Allergenicity of MBT by the Guinea-Pig Maximization Test (data from Magnusson and Kligman, 1969)

Induction		Challenge		
Intradermal Concentration in Adjuvant %	Topical Concentration in Petrolatum %	Topical Concentration in Petrolatum %	Sensitization Rate	Grade
1	25	15	8/20	III (Moderate)

g. Teratogenicity

No data are available.

h. Mutagenicity

A single report, abstracted from the Russian literature, described studies in fruit flies on the mutagenic and morphologic effects of several rubber additives (Revazova, 1968). These results are presented in Table 35. Although the use of Drosophila melanogaster (fruit fly) as a mutagenic

Table 35. Mutagenic Activity of Several Rubber Additives by Feeding to Fruit Flies (from Revazova, 1968)

Substance	Concentration (mg/cc)	Number of days	% Mutations
2-Mercaptobenzothiazole	20-40	8-10	2.5±0.49
N-Oxydiethylene-2-benzothiazolesulfenamide	20-40	8-9	2.9±0.38
N-Cyclohexyl-2-benzothiazolesulfenamide	20-40	12-14	1.4±0.47
N,N-Dicyclohexyl-2-benzothiazolesulfenamide	20-40	12-16	0.4±0.26
N,N-Hexamethylene-2-benzothiazolesulfenamide	20-40	10-13	2.1±0.54
3-(Diethylaminomethyl) benzothiazole-2-thione	20-40	8-12	2.6±0.41

detection system presents several distinct advantages (short generation time, large chromosomes, small chromosome number), a number of drawbacks must also be considered. Large differences between man and fruit flies in such areas as foreign compound metabolism, physiologic processes and life span will seriously hinder the direct extrapolation of experimental results from Drosophila to man. Furthermore, the fruit fly, due to its relatively short life span, is unsuitable for mutagenicity testing aimed at chronic exposure to environmental chemicals. Interpretation of the above data is especially difficult since it is not possible to determine the objectivity of the results presented due to the lack of comparison scores between treated and control groups. This factor is very important since it is difficult to maintain constant environmental control over critical variables such as temperature, humidity and nutrition. The desirability of mutagenic testing of MBT type rubber accelerators in higher test organisms is obviously very high if we wish to confirm their mutagenic activities as indicated by the above results. Furthermore, although a large number of mutagens also possess carcinogenic activity, several MBT derivatives have been screened as possible carcinogens and found to be negative.

i. Carcinogenicity

The tumorigenicity of MBT and several related compounds was tested by daily oral administration to both sexes of two hybrid strains of mice starting at the age of 7 days (Innes et al., 1969). The compounds were administered at the maximal tolerated doses as outlined in Table 36. Eighteen mice of each sex of each strain were treated by stomach tube during days 7 to 28 of age and thereafter given the chemical mixed in the diet through the remainder of the 18 month test period. None of the chemicals tested in this study caused a

Table 36. Administration of MBT and Several Derivatives to Mice (data from Innes _et_ _al_., 1969)

Substance	Daily Dosage[a] (mg/kg)	Vehicle[a]	ppm[b]
2-Mercaptobenzothiazole	100	0.5% gelatin	323
2,2'-Dithiobisbenzothiazole	464	0.5% gelatin	1577
Zinc salt of 2-mercaptobenzothiazole	1000	0.5% gelatin	3385
N-Cyclohexyl-2-benzothiazolesulfenamide	215	0.5% gelatin	692
N-Oxydiethylene-2-benzothiazolesulfenamide	464	0.5% gelatin	1492

[a] Used during stomach-tubing period only (7-28 days of age)

[b] Dosage in diet, given ad libitum (after 28 days of age)

significant increase in tumor incidence above control animal values.

A derivative of benzothiazole has been identified by Hadidian _et_ _al_. (1968) as having definite tumorigenic activity. This compound, 2-(4-dimethylaminostyryl)benzothiazole, is related to several quinoline analogs which are also known carcinogens. Administration of the compound was made by gastric intubation at dose levels of 0.3 to 100 mg/kg/day to male and female rats, and was performed five times per week for a total of 260 individual doses in one year. They found that tumors developed at all dose levels and consisted mainly of testicular interstitial cell tumors in males and mammary fibroadenomas in females.

Two benzothiazole derivatives with potent hepatocarcinogenic activity in male rats were reported by Brown and Sanchorawala (1968). The most active compound tested, N,N-dimethyl-p-(6-benzthiazolylazo)aniline, when fed in the diet at 0.03% gave a tumor incidence of 5/10 in one month and 10/10 in two months. N,N-dimethyl-p-(7-benzothiazolylazo)aniline gave a 10/10 incidence in three months at the 0.03% dietary level.

When benzothiazole was tested for carcinogenicity in rats by adding it to the diet, Brown (1963) reported that no activity was demonstrated after six months of feeding.

j. Other Chronic Effects Studies

The results of a two-year feeding study using dogs and rats exposed to MBT have been reported by Lehman (1965). The formulation employed was a mixture of 2.4% MBT and 27.6% dimethyldithiocarbamate. Male and female rats were fed the mixture for two years in their diet at 500, 1580, and 5000 ppm, which represents 12, 37.9 and 120 ppm, respectively, of MBT. Male and female dogs were treated with the same mixture and dietary concentrations, but for a period of one year only. In both the rat and dog studies, there was no significant effect on mortality at any of the dosages employed. In addition, no deleterious effects were seen for body weight gain, hematologic examinations, or histopathology. Furthermore, in the rat, no cumulative effect could be demonstrated on reproduction and lactation through the F2 generation, and no demonstrable storage was found in the liver, kidney, or spleen when rats were fed the mixture at 10,000 ppm for one week. Based on these results, a no-effect level for MBT in the diet was set at 120 ppm for both rats and dogs.

k. Behavioral Effects

No data are available.

l. Possible Synergisms

No data are available.

3. Effects on Other Vertebrates

A single study has been encountered (Pickering and Henderson, 1966) which presents the results of acute static toxicity studies in fish for the MBT-containing fungicides Vancide 51Z and the manganese salt of Vancide 51. Three species of fish were tested at five different concentrations, and mortalities were recorded at 24-, 48-, and 96-hour intervals. Table 37 presents the TL_m values (a statistical estimate of the concentration of a material in water that kills 50 percent of the test species) obtained in a number of bioassays, with ten fish being used for each test concentration. As the results in Table 37 indicate, neither Vancide 51Z nor the manganese salt of Vancide 51 is significantly more toxic at 96 hours than at 24 hours. This observation suggests that cumulative toxicity is not a major factor in the mortality produced by these compounds. Furthermore, a comparison of the acute toxicity of both substances when tested in either hard or soft dilution water did not reveal any major differences. It is not possible to determine from this study to what extent the individual components of the test formulation contributed to the overall toxic response. Acute toxicity studies on fish exposed to pure MBT have not been found.

4. Effects on Invertebrates

One report, read in abstract, (Miyaki and Enomoto, 1957) has stated that MBT is a powerful molluscacide against Oncomelania nosophora. No further reports on toxicity to invertebrates have been encountered.

5. Effects on Plants

a. Fungi

Many heterocyclic mercaptan compounds have been found to be active fungicides. More specifically, MBT was shown to be active against smut

Table 37. Summary of the Acute Toxicity of Vancide Formulations to Several Species of Fish (data from Pickering and Henderson, 1966)

Compound	Solvent or Carrier	Dilution Water[a]	Test Fish	24-hour			48-hour			96-hour		
				TL_m^b	Conf. limits		TL_m^b	Conf. limits		TL_m^b	Conf. limits	
Vancide 51Z (Dispersion 50% active Ziram-Zn salt of Di-Methyldithiocarbamic acid 46%; Zinc salt of Mercaptobenzothiazole 4%)	Water	S	Fatheads	.41	.31	.52	.35	.24	.45	.35	.24	.45
	Water	S	Bluegills	.85	.74	1.1	.85	.74	1.1	.85	.74	1.1
	Water	S	Guppies	.59	.51	.70						
	Water	H	Guppies	.51	.41	.67						
Manganese Salt of Vancide 51, 85% Active	Water blend	S	Fatheads	.91	.73	1.2	.83	.66	1.1	.83	.66	1.1
	Water blend	H	Fatheads	.97	.77	1.3	.75	.58	.95	.71	.55	.90

[a] S = soft water, H = hard water

[b] mg of formulation/liter and 95% confidence limits

and bunt of wheat, carnation rust, mildew on leather and textiles, and mycoses of humans (Owens, 1969). It was suggested that the thiol group of MBT is essential for toxicity, since neither benzothiazole nor 2-chlorobenzothiazole is an active fungicide.

Several commercial fungicide formulations incorporate MBT as an active ingredient, but it is rarely used alone. The effectiveness of MBT as a primary fungicide was tested by Chatterjee *et al*., (1961). An assay system was employed whereby the ability of MBT to inhibit the germination of spores of *Aspergillus niger* and *Memnoniella echinata* was evaluated. Calculations were made for the percent concentrations, expressed in mg/100 ml, required for 50 and 90 percent inhibition of spore germination (Lc50 and Lc90, respectively). Spore suspensions used for testing were prepared with a density of 50,000 spores/ml. Their results are presented in Table 38.

Table 38. Assessment of Fungicidal Activity (data from Chatterjee *et al*., 1961)

Name of Fungicide	Test Organism	Lc50 (mg/100 ml)	Lc90 (mg/100 ml)
Vulcafor Daw (active ingredient - MBT)	*Aspergillus niger*	0.06035	0.8439
	Memnoniella echinata	0.00605	0.05962

Several additional benzothiazole derivatives have been tested for fungistatic activity by Merkel *et al*., (1963). These chemicals were tested on six strains of yeast-like fungi *Candida albicans*, and six strains of dermatophytes: *Trichophyton gypseum*, *Tr*. *interdigitalis*, *Tr*. *plicatile*, *Tr*. *sulfureum*, *Epidermophyton rubrum*, and *Microsporum fulvum*. Responses to chemical exposure in liquid and solid media were expressed as molar concentrations of the substance required to inhibit growth (Table 39).

Table 39. Action of Benzothiazole Derivatives on Pathogenic Strains of Dermatophytes and Yeast-like Fungi (data from Merkel *et al*., 1963)

	Molar Concentrations Inhibiting Growth	
Compound Tested	Dermatophytes	Yeast-like fungi
2-Methylbenzothiazole	10^{-5}	10^{-5}
2-Methyl-4-chlorobenzothiazole	10^{-5}	10^{-5}
2-Methyl-5-chlorobenzothiazole	10^{-4}	10^{-8}
2-Methyl-6-chlorobenzothiazole	10^{-8}	10^{-8}
2-Methyl-7-chlorobenzothiazole	10^{-7}	10^{-7}

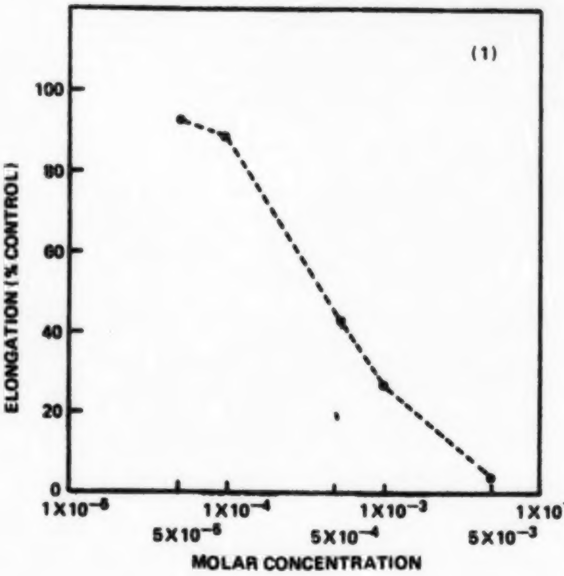
The zinc salt of MBT was tested against strains of *Stemphylium sarcinaeforme* and *Curvularia lunata* (Horsfall and Rich, 1951). The respective concentrations causing germination failure in 50% of the spores were 8.4 and 5.0 ppm.

b. Higher Plants

A number of compounds belonging to the benzazole class (e.g., benzimidazole, benzothiazole, benzotriazole) were evaluated by Klingensmith (1961) for their effects on growth of seedlings, established plants, and plant tissue cultures. Because the primary root of a seedling is highly responsive to chemical modifications, Klingensmith applied benzothiazole and mercapto-S-methylbenzothiazole to germinating cucumber seedlings in order to determine their effects on elongation of the primary root (Table 40). In addition, dose-response curves for repression of root elongation in cucumber seedlings were constructed after treating the plants with benzothiazole (Figure 10). Benzothiazole was also tested on barley to determine its effect on the growth response involving the conversion of endosperm to dry matter in the roots (Figure 11).

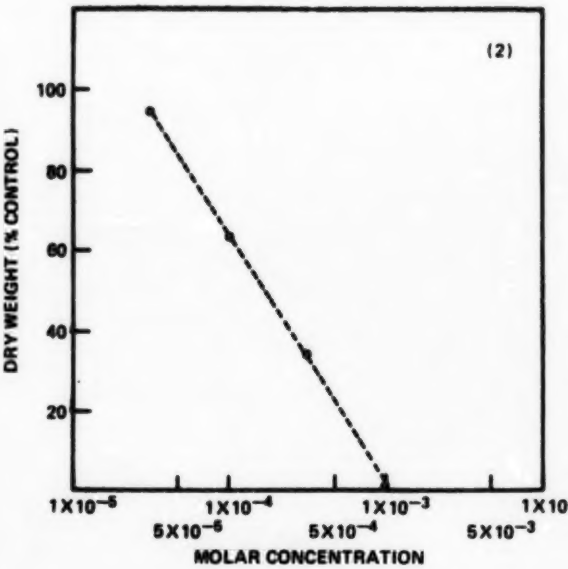
Table 40. Repression of Cucumber Root Elongation by Azoles. Elongation of Primary Root. Cucumber "Early Fortune," 96 hr. at 25°C. (data from Klingensmith, 1961)

Compound	Concentration causing 50% repression
Benzothiazole	5×10^{-4} M
Mercapto-S-methylbenzothiazole	4×10^{-4} M



Seedlings grown in presence of benzothiazole at 25°C in darkness for 96 hours.

Figure 10. Effect of Benzothiazole Upon the Elongation of the Primary Root of Cucumber Seedlings (data from Klingensmith, 1961)



Seedlings grown in aerated deep cultures, 25°C in darkness for six days.

Figure 11. Effect of Benzothiazole Upon Dry Weight of Barley Roots (data from Klingensmith, 1961)

When benzotriazole and benzimidazole were tested for their effects on inhibition of the elongation of cucumber root, they were only about one-tenth as active as benzothiazole. In repressing the growth of barley roots, benzimidazole was only about one-tenth as active as benzothiazole and benzotriazole. The mechanism of action for the inhibition of root elongation was not discovered, but attempts to reverse the repression by adding 5×10^{-4} M adenine were not successful. This result indicates that benzothiazole does not act as a purine anti-metabolite, and therefore inhibition of growth probably does not involve interference with the synthesis of nucleic acid.

Further effects were noted when established plants were exposed to benzothiazole. When bushbeans were treated with 1×10^{-2} M benzothiazole, adventitious roots developed along the lower portion of the stem or hypocotyl. To further explore this phenomenon, a series of plants were subjected to environmental changes around the stem, both with and without the addition of benzothiazole. The treated plants received 25 ml of 1×10^{-2} M benzothiazole applied to the vermiculite, and each treatment was replicated six times with two plants in each container. The results obtained (Table 41) show that benzothiazole was responsible for the formation of adventitious roots from the stem. Increased moisture around the stem, as in deep-planting and when the container top was enclosed with "Saran Wrap," further enhanced the number of roots emerging.

Table 41. Induction of Adventitious Roots in Bushbeans by Root Application of $1x10^{-2}$ M Benzothiazole (18 day old "Black Valentine" Bean Plants, 25 ml. solution applied to the vermiculite) (from Klingensmith, 1961)

Environment Variable	Number of plants with adventitious roots	
	No benzothiazole	Plus benzothiazole
Control	0	7
Deep planting	2[a]	10
Stem wrapped with cotton	0	6
"Saran Wrap" moist chamber	0	11

[a] Each plant had 1 small adventitious root.

When benzothiazole in solutions of $1x10^{-2}$ M or $3x10^{-3}$ M was applied to tomato plants, there was an induction of root primordia to within several inches of the apex in the stem. The application of benzothiazole at $1x10^{-2}$ M to the root medium of tomato also caused dry, dark-brown, sunken lesions to appear on the upper portion of the stem and on the petioles. Several days later, the basal leaves began to turn yellow, and the death of the plant usually resulted in 10 to 14 days.

A series of experiments was also conducted on tobacco stem segments to which had been added benzothiazole at concentrations of $5x10^{-6}$ M, $5x10^{-5}$ M, and $5x10^{-4}$ M in the culture media. Klingensmith (1961) found that in stems treated with $5x10^{-4}$ M benzothiazole, not only were buds produced but root formation was also enhanced. These results were particularly interesting because root formation is dependent upon the ratio of adenine to auxin, with a high concentration of auxin favoring root development. In these experiments, however, exogenous auxin was not added, yet benzothiazole nevertheless was able to induce root growth. Furthermore, at low auxin-high adenine concentrations, bud development is favored. In the benzothiazole-treated tobacco segments, however, sections which produced roots also had more buds than the controls.

Observations that MBT at concentrations of 10^{-4} M would completely inhibit the browning reactions in banana pulp were shown by Palmer and Roberts (1967) to be due to an inhibition of the enzyme banana polyphenoloxidase (PPO). They suggested that MBT inhibition of PPO, a copper metalloenzyme, could be due to interactions between MBT and the enzymically bound copper. This argument is strengthened by the fact that addition of Cu^{++} to the assay system could reduce or completely overcome the inhibition by MBT. These results are consistent with observations by Wang and Mellenthin (1974) who demonstrated that friction discoloration of d'Anjou pears could be prevented by MBT treatment, and was apparently due to inactivation of the PPO of the pear. In higher animals, the ability of MBT to interact with copper metalloenzymes has also been postulated (see Section II-B-2-c, p. 79).

6. Effects on Microorganisms

A large number of benzothiazole derivatives have been synthesized and tested for antibacterial activity, and many of these compounds are very effective bactericidal and bacteriostatic agents. Hundreds of benzothiazole derivatives have been tested as possible chemotherapeutic agents with antibacterial or antiviral effectiveness, including benzothiazole basic ethers (Cossey et al., 1966), substituted alkyl (2-benzothiazolylthio) acetates and alkyl (6-x-2-benzothiazolylthio) formates (Mikulasek et al., 1974), and various substituted chlorobenzothiazoles (Logemann et al., 1961).

In a study conducted by Foltinova and Blockinger (1970), it was demonstrated that MBT, MBT thiolates together with metals, and 6-nitro-2-mercaptobenzothiazole are all effective against G+ and G- bacterial strains. The inhibitory concentrations of MBT and its derivatives on bacterial growth are presented in Table 42. Table 43 summarizes the effectiveness of MBT against a number of mycobacterial strains.

Recent studies were conducted by Aktulga (1972) in which a number of ingredients found in pharmaceutical rubber closures were tested for inhibitory action on microorganisms. Among these substances were MBT and 2,2'-dithiobisbenzothiazole. Bacteria culture media was prepared in petri dishes and inoculated with young broth cultures of several bacterial strains. Both compounds were sterilized and placed on the agar plates at a concentration of either 2 or 5 mg per plate. The diameters of the zones of growth inhibition produced by the two chemicals are presented in Table 44.

Table 42. Effect of MBT and Several Derivatives on _Staphylococcus aureus_ and _Escherichia coli_ (from Foltinova and Blockinger, 1970)

Compound	*Staphylococcus aureus*						*Escherichia coli*					
	Growth of Control	I	II	III	IV	V	Growth of Control	I	II	III	IV	V
MBT	+ + +	–	–	–	+	+ +	+ + +	–	–	–	+	+ +
6-NO_2-2-MBT	+ + +	–	–	–	–	+	+ + +	–	–	–	–	+
$2Cu^{+2}$-MBT	+ + +	–	–	–	–	+ +	+ + +	–	–	–	–	+ +
2Ag-MBT	+ + +	–	–	–	–	+ +	+ + +	–	–	–	–	+ +
$2Hg^{+2}$-MBT	+ + +	–	–	–	–	+	+ + +	–	–	–	–	+
$2Pb^{+2}$-MBT	+ + +	–	–	–	–	+ +	+ + +	–	–	–	+	+ +
$2Bi^{+3}$-MBT	+ + +	–	–	–	–	+	+ + +	–	–	–	+	+ +

I = 1000 μg/ml
II = 500 μg/ml
III = 250 μg/ml
IV = 100 μg/ml
V = 50 μg/ml

Table 43. Antimycobacterial Effects of 2-Mercaptobenzothiazole (data from Foltinova and Blockinger, 1970)

Organism	Undefined Media			Synthetic Media		
	Growth of Control	Complete Inhibition (μg/ml)	Partial Inhibition (μg/ml)	Growth of Control	Complete Inhibition (μg/ml)	Partial Inhibition (μg/ml)
M. tuberculosis- H_{37} Rv	+ + + +	250	100 +	+ + + +	100	50 +
M. tuberculosis- H_{37} Rv IR (isonicotyl hydrazide resistant variant)	+ + + +	500	250 +	+ + + +	250	100 +
M. tuberculosis- H_{37} Rv SR (streptomycin resistant variant)	+ + + +	500	250 +	+ + + +	250	100 +
BCG (attenuated strain of *M. tuberculosis*)	+ + + +	100	50 +	+ + + +	50	25 +
M. bovis	+ + + +	250	100 +	+ + + +	100	50 +
M. avium	+ + + +	500	250 +	+ + + +	250	100 +
M. kansasii	+ + + +	500	250 +	+ + + +	250	100 +
M. phlei	+ + + +	>1000	1000 + + +	+ + + +	>500	500 + + +

Table 44. Antibacterial Effect of MBT and 2,2'-Dithiobisbenzothiazole (from Aktulga, 1972)

		Test Organism			
Compound	Amount	*Staphylococcus aureus* zone diameter (mm)	*Escherichia coli* zone diameter (mm)	*Corynebacterium diptheriae* zone diameter (mm)	*Pseudomonas aeruginosa* zone diameter (mm)
MBT	2 mg	1	1	4	–
	5 mg	2	2	large	–
2,2'-Dithiobisbenzothiazole	2 mg	–	–	2	–
	5 mg	–	–	2	–

As indicated by Table 44, MBT is strongly inhibitory toward *Corynebacterium diptheriae* and considerably less effective against the other bacterial tribes tested. Antibacterial properties for 2,2'-dithiobisbenzothiazole could be demonstrated only against *Corynebacterium diptheriae* in this study.

7. *In Vitro* and Biochemical Studies

A cell culture evaluation of the toxicity of MBT and 2-(2-hydroxyethylmercapto) benzothiazole (HMBT) was conducted by Guess and O'Leary (1969) using the NCTC-929, strain L mouse fibroblast cell line (L-929) and 10-day chick embryo cells. Test plates were made by overlaying confluent cell lines with nutrient agar and staining with a vital dye. Toxicity was evident as an all-or-none response when the killed cells released the vital stain to create clear zones around the test material. Any zone of dead cells surrounding the test material was regarded as a positive response, regardless of its size. Table 45 summarizes the results from testing the pure compounds, and various oil solutions and saturated aqueous solutions of MBT and HMBT.

Table 45. Cell Culture Evaluation of MBT and HMBT (from Guess and O'Leary, 1969)

	MBT[a]		HMBT[a]	
Sample	L cells	C.E. cells	L cells	C.E. cells
Pure compound	Pos.	Neg.	Pos.	Pos.
SSS[b]	Neg.	Neg.	Pos.	Pos.
SSS, 1:2 dilution	Neg.	Neg.	Pos.	Pos.
SSS, 1:4 dilution	Neg.	Neg.	Neg.	Neg.
Oil suspensions				
12 mg/ml	Pos.	Pos.	Pos.	Pos.
6 mg/ml	Pos.	Pos.	Pos.	Neg.
3 mg/ml	Pos.	Neg.	Pos.	Neg.
1.5 mg/ml	Neg.	Neg.	Neg.	Neg.
Oil control	Neg.	Neg.	Neg.	Neg.
Saline control	Neg.	Neg.	Neg.	Neg.

[a] Pos. = positive, toxic response; Neg. = negative, nontoxic response.

[b] SSS = saturated saline solution. SSS for MBT = 0.5 mg/ml; SSS for HMBT = 0.8 mg/ml.

Judging by the above results, the authors felt that MBT and HMBT should be considered to have a fairly low order of toxicity, based on the unusally high sensitivity to toxicants of isolated cells in culture. Where cellular damage occurred, it was generally evident as cellular irritation with HMBT treatment, and as vacuolization with MBT treatment.

IV. Regulations and Standards

A. Current Regulation

Regulation and control over derivatives of MBT are provided under several different authorities. Because this group of compounds is involved in a number of applications, product control at the federal level is varied. Effluent control, on the other hand, is exercised under basically the same authority for all these chemicals.

The use of MBT as an agricultural fungicide is regulated under the Federal Environmental Pesticide Control Act of 1972, which has revised the Federal Insecticide, Fungicide, and Rodenticide Act of 1947 (7 U.S.C. 135-135k). Tolerances for pesticide chemical residues in or on raw agricultural commodities have been established under the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 346a). Specific tolerances for MBT have been stated in the Federal Register (22:9258, November 28, 1957). A tolerance of 0.1 ppm was established for MBT, calculated as 2,2'-dithiobisbenzothiazole, in or on apples.

A tolerance has also been established for residues of 2-(thiocyanomethylthio)benzothiazole under the Federal Food, Drug, and Cosmetic Act as detailed in 40CFR 180.288. Tolerances allow for residues of 0.1 ppm in or on barley, corn, cotton forage, cottonseed, oats, rice, safflower, sorghum, sugarbeets, and wheat.

By amendment to the Federal Food, Drug, and Cosmetic Act, the rubber accelerator 1,3-bis(2-benzothiazolylmercaptomethyl)urea is permitted as a component of rubber articles intended for repeated use in contact with food (Federal Register 30:3207, March 9, 1965) and as a component of food-packaging adhesives (Federal Register 30:7386, June 4, 1965).

In a report abstracted from the Russian literature, Vaisman *et al.* (1973) have recommended that undissolved MBT and MBTS be absent from waste waters.

The transportation of hazardous materials by rail and highway is regulated by the Hazardous Materials Regulation Board of the Department of Transportation. In January, 1974, the Department of Transportation proposed extensive changes in the rules governing the transport of hazardous chemicals, especially by air. These changes, published in the *Federal Register* (January 24, 1974) listed a large number of chemical substances. However, no derivatives of MBT are included on this list or classified as being hazardous.

B. Concensus and Similar Standards

Limits have been established for maximum permissible exposure to hazardous chemicals by several agencies. Threshold limit values (TLV's) for chemicals in the workroom environment have been established by the American Conference of Governmental Industrial Hygienists. These TLV's, which are revised and updated each year, do not, however, include MBT or any of its derivatives.

Exposure limits for hazardous substances have also been set by the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health. Their list of standards which has been published in the *Federal Register* (October 19, 1972) does not include any derivatives of MBT.

V. Summary and Conclusions

Mercaptobenzothiazole compounds are important commercial chemicals which are produced in considerable quantities. There are at least sixteen compounds which have the 2-mercaptobenzothiazole moiety and are produced in commercial quantities. The major commercial chemicals include sodium 2-mercaptobenzothiazole (NaMBT), which is used mostly for anticorrosion applications; mercaptobenzothiazole (MBT), 2,2'-dithiobisbenzothiazole (MBTS), zinc 2-mercaptobenzothiazole (ZMBT), N-cyclohexyl-2-benzothiazolesulfenamide (CBS), and a number of other sulfenamide derivatives, which are used almost exclusively as vulcanization accelerators. In general, the compounds are chemically stable, water insoluble, solids which will decompose by free radical mechanisms at vulcanization temperatures to form a variety of compounds, including the parent compound, benzothiazole. The sodium salt is somewhat unusual in that it is water soluble (the other salts are insoluble).

Approximately 100 million pounds of MBT compounds are produced annually. Individual production figures are available for NaMBT (11.9 million pounds - 1973), CBS (4.6 million pounds - 1974), MBT (6.1 million pounds - 1974), MBTS (20.7 million pounds - 1974), and ZMBT (4.0 million pounds - 1972).

The sodium salt (NaMBT) is used as a corrosion inhibitor in water based cooling systems and as a chemical intermediate for some of the other MBT derivatives. Approximately 3 million pounds per year is consumed in automobile cooling systems, and since antifreeze solutions are frequently changed, the release to the environment is probably quite substantial. The quantity lost from other stationary cooling systems (~ 3 million pounds per year) may also be quite high, depending upon how frequently the coolant is changed.

The largest use of MBT compounds is for vulcanization accelerators. These chemicals are added at concentrations of 0.5 - 1.5% before vulcanization, in order to allow the process to take place in a reproducible and uniform fashion at lower temperatures. It appears that substantial amounts of these chemicals may be released to the environment during use in rubber processing and in the use and disposal of rubber products. MBT, MBTS, and benzothiazole have all been detected in effluents from rubber manufacturing plants in the U.S. and USSR. The quantities lost are unknown (0.027 ppm and 0.049 ppm have been detected in the discharge from a tire plant treatment pond), but the rubber manufacturing capacity in the U.S. is approximately 7,500 million pounds, so the quantities could be substantial.

Approximately 1.2 billion pounds of rubber dust are worn from automotive tires each year in the U.S., which amounts to 12 million pounds of MBT compounds (including MBT, MBTS, and benzothiazole) being released each year, based on an average of 1% accelerator in the rubber. The accelerators are probably leached out of this dust by water in the environment, since it has been shown that accelerators can be rapidly dissolved out of rubber products with distilled water. MBT compounds may also be leached out of rubber products that are discarded in garbage dumps, landfills, or waterways.

Very little is known about the environmental fate of MBT compounds. No experimental data on persistence or bioaccumulation are available. Calculations of bioaccumulation potential based upon physical properties would suggest that the compounds probably do not concentrate in higher food chain organisms. The monitoring data provide little clarification of the question of environmental fate. So far, the following compounds have been found in raw or drinking water: benzothiazole, 2-methylbenzothiazole, and 2-thiomethylbenzothiazole. However, in most cases, the analytical procedure and concentrations have not been mentioned and the chemical nomenclature is confusing.

An accumulation of direct evidence and a cautious extrapolation of experimental data indicate that MBT and its derivatives may influence several important biologic parameters in all living organisms. Principally, the actions of MBT derivatives in human and animal systems may involve at least three possible effects: (1) the production of allergic contact dermatitis, (2) action on the central nervous system, and (3) inhibition of certain metalloenzymes which contain copper.

The results of acute toxicity determinations, although somewhat variable and markedly influenced by route of administration, demonstrate that single dose exposure and the capacity to produce immediate death is not the major threat to health posed by these compounds. Indeed, it may be concluded that the acute toxic hazard of MBT derivatives when administered by the routes of probable environmental exposure (e.g., oral, dermal, inhalational) is minimal. Among the chemicals of the benzothiazole class, unsubstituted benzothiazole and several of the 2-amino- and benzene ring-substituted derivatives are considerably more toxic than the rubber accelerator chemicals containing a 2-position sulfur linkage.

The production of allergic contact dermatitis by exposure to MBT in domestic situations has occurred with relatively high frequency. However, there is little evidence of occupational disease which can be attributed solely to contact with MBT or its derivatives. Both animal and man have been shown to develop sensitivity to MBT when applied to the skin. The dermatitic reaction elicited by MBT, while not particularly severe, occurs with enough frequency to warrant classifying MBT as one of the most common contact allergens in use today. Exposure of humans to this substance is accomplished almost exclusively through its use as a component in rubber products. In addition, where sensitivity to MBT has been achieved in man, cross-sensitivity may also be shown to other chemicals containing the MBT moiety.

The potent action on the central nervous system of numerous derivatives of benzothiazole, including MBT, has only been shown to occur in animals. These effects can be manifested as either a central stimulation (convulsions) or as selective depression (flaccid paralysis, mental depression, lack of spontaneous motor activity). The mechanism of these actions has not been investigated, nor have studies been performed to determine the extent of transport and accumulation of benzothiazole compounds in tissues of the central nervous system.

The effects of benzothiazole derivatives on central nervous system function may well be due, in part, to their interaction with specific enzymes. By acting as a copper chelating agent, MBT was shown in mice to inhibit a metalloenzyme which is responsible for the conversion of dopamine to noradrenaline (an important neurohumoral transmitter substance). Symptoms of central nervous system depression could be correlated with levels of noradrenalin in the brain as influenced by MBT exposure. These results are clearly suggestive of the need to investigate other benzothiazole and MBT derivatives with respect to their potential role in disruption of enzyme function.

Feeding studies in mice have been conducted on benzothiazole, MBT, and several MBT type rubber accelerators which demonstrated that these compounds do not appear to present a significant carcinogenic threat. However, limited data from mutagenesis assays in the fruit fly suggest that several MBT compounds may possess mutagenic properties. Additional testing using a more reliable system (e.g., Ames assay) will be necessary to more clearly predict possible genetic damage or other reproductive hazards in humans.

In summary, it appears that substantial quantities of MBT derivatives and benzothiazole are being released to the environment. Their environmental fate is unknown, and the available monitoring data are not detailed enough to suggest the degree of contamination. More information in these areas is needed before a definitive assessment of the environmental hazard associated with the commercial use of MBT compounds is possible.

REFERENCES

Abdulian, D.H., Martin, W.R. and Unna, K.R. (1960), "Effects of Central Nervous System Depressants on Inhibition and Facilitation of the Patellar Reflex", Arch. Int. Pharmacodyn., 128, 169-86.

Aktulga, A. (1971a), "Identification of the Leached Ingredients from Rubber Closures by Spectrophotometric Analysis", Eczacilik Bul., 13(4), 49-56.

Aktulga, A. (1971b), "Identification of the Leached Ingredients from Rubber Closures with Gel Filtration and Quantitative Estimation of 2-Mercaptobenzothiazole", Eczacilik Bul., 13(5), 74-80.

Aktulga, A. (1972), "Toxicity of Rubber", Eczacilik Bul., 14(3), 35-43.

Alaimo, R.J., Pelosi, S.S., Hatton, C.J. and Gray, J.E. (1974), "Antiparasitic Thiocyanatobenzothiazoles", J. Med. Chem., 17(7), 775-6.

Allen, C.F.H. (1966), "Heterocyclic Compounds", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 10, 916.

American Cyanamid Co. (1970), "MBT Product Bulletin".

American Cyanamid Co. (1971), "TBBS Accelerator Product Bulletin".

American Cyanamid Co. (1973), "DIBS Accelerator Product Bulletin".

American Cyanamid Co. (1975), Personal Communication, Wayne, New Jersey.

Anon. (1973a), "Chemical Profile - Aniline", Chemical Marketing Reporter, Dec. 31.

Anon. (1973b), "Are You Drinking Biorefractories Too?", Environ. Sci. Technol., 7, 14.

Anon. (1974), "Chemical Profile - SB Rubber", Chemical Marketing Reporter, June 24.

Anon. (1975a), "Marketing: Sticky Debate", Time, (Dec. 15), 60.

Anon. (1975b), "Chemical Profile - Carbon Disulfide", Chemical Marketing Reporter, June 16.

Baer, R.L., Ramsey, D.L. and Biondi, E. (1973), "The Most Common Contact Allergens", Arch. Dermatol., 108, 74-78.

Bauer, M. (1972), "The Environment and the Woman", Woman Physician, 27(1), 16-20.

Berg, G.L. (1974), Farm Chemicals Handbook, Meister Publishing Co., Willoughby, Ohio, p. D204.

Blank, I.H. and Miller, O.G. (1952), "A Study of Rubber Adhesives in Shoes as the Cause of Dermatitis of the Feet", J. Am. Med. Assoc., 149, 1371-1373.

Bonnevie, P. and Marcussen, P.V. (1944), "Rubber Products as a Widespread Cause of Eczema: Report of 80 Cases", Acta dermat.-venereol., 25, 163-178.

Bowes, J.H., Cater, C.W. and Taylor, J.E. (1970), "Substantive Fungicides", J. Amer. Leather Chem. Ass., 65(2), 85-96.

Brooks, L.A. (1963), "The Chemistry of Thiazoles and Dithiocarbamates as Antioxidants", Rubber Chem. Technol., 36(4), 887-910.

Brown, E.V. (1963), "Carcinogenic Activity of Analogues of p-Dimethylaminoazobenzene", Acta. Un. Int. Cancr., 19, 531-533.

Brown, E.V. and Sanchorawala, C.J. (1968), "Carcinogenic Activity of Analogs of p-Dimethylaminoazobenzene. VI. Activity of the Benzimidazole and Benzthiazole Analogs", J. Med. Chem., 11, 1074-5.

Burnham, A.K., Calder, G.V., Fritz, J.S., Junk, G.A., Svec, H.J. and Vick, R. (1973), "Trace Organics in Water: Their Isolation and Identification", J. Amer. Water Works Assoc., 65, 722.

Calnan, C.D. and Sarkany, I. (1959), "Studies in Contact Dermatitis. IX. Shoe Dermatitis", Trans. St. John's Hosp. derm. Soc., Lond., 43, 8.

Campbell, R.H. and Wise, R.W. (1973), "Sulfenamides by Catalytic Oxidation of Amines", U.S. Patent No. 3,737,431, 6 pp.

Catalona, W.J., Taylor, P.T. and Chretien, P.B. (1972), "Quantitative Dinitrochlorobenzene Contact Sensitization in a Normal Population", Clin. Exp. Immunol., 12(3), 325-33.

Chakravarti, S. and Sircar, A.K. (1965), "Amperometric Determination of Sulfenamide-Type Rubber Accelerators", Trans. Inst. Rubber Ind., 41(6), T221-T236.

Chatterjee, P.K., Banerjee, D. and Sircar, A.K. (1960), "Determination of Thiazole Type of Rubber Accelerators by Amperometric Titration", Trans. Inst. Rubber Ind., 36, 65-9.

Chatterjee, M.G. Ranganathan, S.K., Saxena, B.B.L. and Sengupta, S.R. (1961), "Rapid Method for the Assessment of Water Soluble and Insoluble Fungicides and the Results of Examination of Certain Fungicides", Defence Sci. J., 11, 170-5.

Chemical Marketing Reporter (1975), "Current Prices of Chemicals and Related Materials", Dec. 8.

Chesick, J.P. and Donohue, J. (1971), "Molecular and Crystal Structure of 2-Mercaptobenzothiazole", Acta Crystallogr., Sect. B, 27(7), 1441-4.

Christensen, H.E. and Luginbyhl, T.T. (1974) Toxic Substances List, 1974, Nat'l. Inst. for Occup. Safety and Hlth.

Collins, H.H. and Higgins, R.I. (1959), "The Corrosion of Road Vehicle Engines by Anti-Freeze Solutions", J. Res. & Dev. Brit. Cast Iron Res. Assoc., 7, 667.

Colucci, D.F. and Buyske, D.A. (1965), "The Biotransformation of a Sulfonamide to a Mercaptan and to Mercapturic Acid and Glucuronide Conjugates", Biochem. Pharmacol., 14, 457-466.

Cooper, H.B.H. and Mensing, C.E. (1951), "Mercaptobenzothiazole", U.S. Patent No. 2,567,146, Sept. 4.

Cossey, H.D., Gartside, R.N. and Stephens, F.F. (1966), "The Antimicrobial Activity of Benzothiazole Basic Ethers and Related Compounds", Arzneimittel-Forsch, 16(1), 33-40.

Coran, A.Y. (1965), "Vulcanization. VII. Kinetics of Sulfur Vulcanization of Natural Rubber in Presence of Delayed-Action Accelerators", Rubber Chem. Technol., 38(1), 1-14.

Cronin, E. (1966), "Shoe Dermatitis", Brit. J. Dermatol., 78, 617-625.

Dannis, M.L. (1975), "Rubber Dust from the Normal Wear of Tires", Proc. Environmental Aspects of Chemical Use in Rubber Processing, March 12-14, EPA-560/1-75-002.

Darby, R.T. and Kempton, A.G. (1962), "Soil Burial of Fabrics Treated with Minimal Concentrations of Fungicides", Textile Res. J., 32, 548-52.

Davis, L.N., Santodonato, J., Howard, P.H. and Saxena, J. (1976), "Investigation of Selected Potential Environmental Contaminants: Benzotriazole," Syracuse Research Corporation, Prepared for the U.S. Environmental Protection Agency, Contract No. 68-01-2987 (In press).

Davis, P.A. (1930), "Toxic Substances in the Rubber Industry. XI. Mercaptobenzolthiazol", Rubber Age, 27, 249-250.

Diamantatos, A. (1973a), "Solvent Extraction of Platinum-Group Metals and Gold with 2-Mercaptobenzothiazole", Anal. Chim. Acta., 66(1), 147-51.

Diamantatos, A. (1973b), "New Solvent Extraction Scheme for the Separation of Platinum, Palladium, Rhodium, and Iridium", Anal. Chim. Acta., 67(2), 317-23.

Dogadkin, B.A., Fel'dshtein, M.S. and Belyaeva, E.N. (1961), "Chemical Interaction and Mechanism of Action of Binary Systems of Vulcanization Accelerators", J. Polymer Sci., 53, 225-31.

Domino, E.F., Unna, K.R. and Kerwin, J. (1952), "Pharmacological Properties of Benzazoles. I. Relationship Between Structure and Paralyzing Action", J. Pharmacol. Exptl. Therap., 102, 486-497.

Doull, J., Plzak, V. and Brois, S.J. (1962), "A Survey of Compounds for Radiation Protection", U.S. Nat. Tech. Inform. Serv., AD-277689.

Eisen, H.N., Orris, L. and Belman, S. (1952), "Elicitation of Delayed Allergic Skin Reactions with Haptens: The Dependence of Elicitation on Hapten Combination with Protein", J. Exp. Med., 95, 473-487.

Eisen, H.N. and Tabachnick, M. (1958), "Elicitation of Allergic Contact Dermatitis in the Guinea Pig: The Distribution of Bound Dinitrobenzene Groups Within the Skin and Quantitative Determination of the Extent of Combination of 2,4-Dinitrochlorobenzene with Epidermal Protein In Vivo", J. Exp. Med., 108, 773-796.

Elkin, E.M. (1969), "Tellurium and Tellurium Compounds", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 19, 771.

Enna, S.J. and Schanker, L.S. (1959), "Drug Absorption from the Lung", Fed. Proc., 28, 359.

Epstein, E. (1973), "Shoe Contact Dermatitis", Arch. Dermatol., 180, 848-849.

Everett, F.G. and Hice, T.L. (1974), "Contact Stomatitis Resulting from Use of Orthodontic Rubber Elastics", J. Am. Dental Assoc., 88, 1030-1.

Evstifeev, M.M., Ivanova, Z.I., Kimstach, V.A. and Gavrilko, Y.M. (1971), "Efficient System for Analytical Monitoring of Toxic Substances in Waste Waters of the Chemical Process Industry", Novye Metody Khim. Anal. Mater., 2, 84-7.

Fiorenza, A., Bonomi, G. and Piacentini, R. (1963), "Spectrophotometry and Chromatography for Identification of Antioxidants and Accelerators in Rubber Mixtures", Rubber Chem. Technol., 36(4), 1119-28.

Fisher, A.A. (1973), Contact Dermatitis, 2nd Ed., Lea and Febiger, Philadelphia.

Foltinova, P. and Blockinger, G. (1970), "Antibacterial Effect of 2-Mercaptobenzothiazole and Its Derivatives", Biologia (Bratislava), 25(3), 175-80.

Fregert, S. (1969), "Cross Sensitivity Pattern of 2-Mercaptobenzothiazole (MBT)", Acta. derm.-venereol., 49, 45-48.

Gaul, L.E. (1957), "Results of Patch Testing with Rubber Antioxidants and Accelerators", J. Invest. Dermatol., 29, 105-10.

Gell, P.G.H. and Godfrey, H.P. (1974), "Studies on Cell Separations in Delayed Hypersensitivity", Monogr. Allergy, 8, 89-99.

Goodrich Chemical Company (1975), Technical Data Sheets: Goodrite MBT, Goodrite ZMBT, Goodrite OBTS, Goodrite CBTS, Goodrite MBTS.

Grushevskaya, N.Y. (1974), "Determination of Some Benzothiazoles as Accelerators of Vulcanization During Hygienic-Chemical Studies of Rubbers", Kauch. Rezina, (1974), 46-7.

Guess, W.L. and O'Leary, R.K. (1969), "Toxicity of a Rubber Accelerator", Toxicol. Appl. Pharmacol., 14(2), 221-31.

Guruswamy, S. and Jayakrishnan, P. (1972), "Electrophoretic Painting Mild Steel. Castor Oil-Maleic Anhydride Resins", Metal Finish., 70(1), 42-5.

Hadidian, Z., Fredrickson, T.N., Weisburger, E.K., Weisburger, J.H., Glass, R.M. and Mantel, N. (1968), "Tests for Chemical Carcinogens. Report on the Activity of Derivatives of Aromatic Amines, Nitroamines, Quinolines, Nitroalkanes, Amides, Epoxides, Aziridines, and Purine Antimetabolites", J. Nat. Cancer Inst., 41(4), 985-1024.

Hardman, A.F. (1956), "Sulfenamides", U.S. Patent No. 2,772,278, Nov. 27.

Herrmann, W.P. and Schulz, K.H. (1960), "Substances Used in the Rubber Industry As a Cause of Eczema", Dermatologica, 120, 127-138.

Hilton, A.S. and Altenau, A.G. (1973), "Mass Spectrometric Identification of 2-Mercaptobenzothiazole Sulfenamide Accelerators in Rubber Vulcanizates", Rubber Chem. Technol., 46(4), 1035-43.

Hindson, T.C. (1966), "Studies in Contact Dermatitis. XVI. Contraceptives", Trans. St. John's Hosp. Derm. Soc., Lond., 52, 1.

Horsfall, J.G. and Rich, S. (1951), "Fungitoxicity of Heterocyclic Nitrogen Compounds", Contrib. Boyce Thompson Inst., 16, 313-347.

Horsfall, J.G. (1956), Principles of Fungicidal Action, Chronica Botanica Co., Waltham, Massachusetts, 201-210.

House, J.E., Jr. and Lau, P-S. (1974), "Complexes of Benzotriazole, Benzoxazole, and Benzothiazole with Palladium (II)", J. Inorg. Nucl. Chem., 36(1), 223-4.

Innes, J.R.M., Ulland, B.M., Valerio, M.G., Petrucelli, L., Fishbein, L., Hart, E.R., Pallotta, A.J., Bates, R.R., Falk, H.L., Gart, J.J., Klein, M., Mitchell, I. and Peters, J. (1969), "Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice: A Preliminary Note", J. Nat. Cancer Inst., 42(6), 1101-1114.

Ivanova, V.A. and Shebuev, A.N. (1957), "Synthesis of 2-Mercaptobenzothiazole", J. Appl. Chem. U.S.S.R., 30, 473-9.

Jenkins, D., Snoeyink, V.L., Adrian, G.W., Faust, S.D., Grigoropoulos, S.G., McCabe, L.J., Jr., Okun, D.A., Ongerth, H.J. and Rosen, A.A. (1974), "Organic Contaminants in Water - Committee Report", J. Amer. Water Works Assoc., 66, 682.

Johnson, G.A., Boukma, S.J. and Platz, P.A. (1970), "2-Mercaptobenzothiazole, An Inhibitor of Dopamine β-Hydroxylase", J. Pharm. Pharmacol., 22(9), 710-12.

Jones, M.H. and Woodcock, J.T. (1973), "UV Spectrophotometric Determination of 2-Mercaptobenzothiazole (MBT) in Flotation Liquors", Can. Met. Quart., 12(4), 497-505.

Jones, M.H. and Woodcock, J.T. (1975), "Determination of Mercaptobenzothiazole (MBT) in Flotation Liquors by Solvent Extraction and Ultraviolet Spectrometry", Anal. Chem., 47(1), 11-16.

Jordan, W.P. (1972), "Clothing and Shoe Dermatitis. Recognition and Management", Postgrad. Med., 52(11), 143-148.

Kamiya, T., Teraji, T., Saito, Y., Hashimoto, M., Nakaguchi, O. and Oku, T. (1973), "β-Lactam Antibiotics. I. Novel Conversion of Penicillins Into Cephalosporins", Tetrahedron Lett., 32, 3001-4.

Khan, M.M. and Malik, A.U. (1972), "Interaction of Copper (I) Halides with 2-Mercaptobenzothiazole", J. Inorg. Nucl. Chem., 34(6), 1847-50.

Khullar, I.P. and Agarwala, U. (1975), "Complexes of 2-Mercaptobenzothiazole with Copper (II), Nickel (II), Cobalt (II), Cadmium (II), Zinc (II), Lead (II), Silver (I), and Thallium (I)", Can. J. Chem., 53(8), 1165-71.

Kleiman, M. (1950), "Organic Sulfur Compounds", U.S. Patent No. 2,510,893, June 6.

Kligman, A.M. (1966a), "The Identification of Contact Allergens by Human Assay. I. A Critique of Standard Methods", J. Invest. Dermatol., 47, 369-374.

Kligman, A.M. (1966b), "The Identification of Contact Allergens by Human Assay. II. Factors Influencing the Induction and Measurement of Allergic Contact Dermatitis", J. Invest. Dermatol., 47, 375-392.

Kligman, A.M. (1966c), "The Identification of Contact Allergens by Human Assay. III. The Maximization Test: A Procedure for Screening and Rating Contact Sensitizers", J. Invest. Dermatol., 47, 393-409.

Klingensmith, M.J. (1961), "Effect of Certain Benzazole Compounds on Plant Growth and Development", Am. J. Botany, 48, 40-5.

Kouris, C.S. and Northcott, J. (1963), "Aniline", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 2, 417.

Kowalski, Z. and Bassendowska, E. (1965), "Toxic Effects of Certain Vulcanization Accelerators", Med. Pracy., 16(1), 35-43.

Krapivkina, L.S. and Rotmistrova, G.B. (1969), "Use of Corrosion-Inhibiting Lubricants to Protect Steel and Reinforced Concrete Structures of Chemical Industries", Uch. Zap. Mosk. Gos. Pedagog. Inst., 303, 302-6.

Krivoruchko, F.D. (1972), "Spectrophotometric Determination of Captax (2-Mercaptobenzothiazole) in Air", Gig. Sanit., 12, 99.

Lehman, A.J. (1965), Summaries of Pesticide Toxicity, Assoc. Food and Drug Officials of the U.S., Topeka, Kansas.

Leib, R.I., Sullivan, A.B. and Trivette, C.D., Jr. (1970), "Prevulcanization Inhibitor - The Chemistry of Scorch Delay", Rubber Chem. Technol., 43, 1118.

Litvinchuk, M.D. (1963), "Biological Tests of 2-Mercaptobenzothiazole, Rubber Vulcanization Accelerator", Farmakol. i Toksikol., 26(4), 484-8.

Logemann, W., Almirante, L., Galimberti, S. and DeCarneri, I. (1961), "Influence of Dichloroacetylation on the Antimicrobial Activity of Chloramphenicol Derivatives and of Various Amines", Brit. J. Pharmacol., 17, 286-296.

Lunt, J.C. (1956), "Cyclohexyl Benzothiazoylsulfenamide Vulcanization Accelerator", U.S. Patent No. 2,762,814, Sept. 11.

Magnusson, B. and Kligman, A.M. (1969), "The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test", J. Invest. Dermatol., 52, 268-276.

Malik, A. and Rahmani, F. (1975), "Complexes of Rare Earths with 2-Mercaptobenzothiazole", J. Inorg. Nucl. Chem., 37(6), 1552-3.

Merkel, M., Rokicka, T., Zawadowski, T. and Szuchnik, A. (1963), "Investigations of Compounds with Antimycotic Effectiveness. IV. Mycological Investigations with Some Derivatives of Benzothiazole and Benzofuran", Bull. Acad. Polon. Sci., Ser. Sci. Biol., 11, 449-58.

Metcalf, R.L. and Lu, P.Y. (1973), Environmental Distribution and Metabolic Fate of Key Industrial Pollutants and Pesticides in a Model Ecosystem, U.S. Nat'l. Tech. Inform. Serv., PB Report No 225479.

Miettinen, T.A. and Leskinen, E. (1970), "Glucuronic Acid Pathway", Metabolic Conjugation and Metabolic Hydrolysis. Vol. I, Academic Press, N.Y.

Mikhailov, A.V. (1973), "Biological Effect of Rubber Vulcanization Accelerators During Chronic Experimental Poisoning", Nauchn. Tr., Permsk. Politekh. Inst., 141, 117-19.

Mikulasek, S., Sutoris, V., Foltinova, P., Konecny, V. and Blockinger, G. (1974), "Benzothiazole Compounds. V. Synthesis and Antimicrobial Effects of Some Esters of (2-Benzothiazolylthio)acetic Acid and 6-Substituted (2-Benzothiazolylthio)formic Acid", Chemicke Zvesti, 28(5), 686-92.

Miyaki, K. and Enomoto, T. (1957), "The Inhibition of Tyrosinase by 2-Benzothiazolethiol", J. Pharm. Soc. Japan, 77, 103-5.

Nakagawa, S., Ueki, H. and Tanioku, K. (1971), "Distribution of 2,4-Dinitrophenyl Groups in Guinea Pig Skin Following Surface Application of 2,4-Dinitrochlorobenzene. Immunofluorescent Study", J. Invest. Dermatol., 57(4), 269-77.

Nathan, C.C. (1965), "Corrosion Inhibitors", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 6, 343.

Neely, W.B., Branson, D.R. and Blau, G.E. (1974), "Partition Coefficient to Measure Bioconcentration Potential of Organic Chemicals in Fish", Environ. Sci. Technol., 8(13), 1113-1115.

Nigam, R.S. and Sanyal, B. (1971), "Corrosion of Steel in Aqueous Solutions of Alcohol", Labdev, Part A, 9(1), 63-71.

Niles, R.C., (1976), "Personal communication", Uniroyal Inc., Middlebury, Conn.

Oleffe, J., Nopp-Oger, M.J. and Achten, G. (1972), "European Battery of Skin Tests - Results of 300 Observations", Berufsdermatosen, 20(5), 209-217.

Oosterhof, D. (1972), "Polybutadiene Elastomers", Chemical Economics Handbook, Stanford Research Institute, Menlo Park, California.

Owens, R.G. (1969), "Organic Sulfur Compounds", Fungicides, An Advanced Treatise, Vol. II, Academic Press, N.Y., 161-165.

Palmer, J.K. and Roberts, J.B. (1967), "Inhibition of Banana Polyphenoloxidase by 2-Mercaptobenzothiazole", Science, 157, 200-201.

Parsons, J.S. and Mitzner, S. (1975), "Gas Chromatographic Method for Concentrations and Analysis of Traces of Industrial Organic Pollutants in Environmental Air and Stacks", Environ. Sci. Technol., 9, 1053.

Petrenko, L.P., Yakobi, V.A., Lyashchenko, M.V. and Dubinskaya, T.P. (1975), "Oxidation of 2-Mercaptobenzothiazole by Ozone", Izv. Vyssh. Uchebn. Zaved., Khim. Khim Tekhnol., 18(2), 304-6.

Pickering, Q.H. and Henderson, C. (1966), "Acute Toxicity of Some Pesticides to Fish", Ohio J. Sci., 66(5), 508-13.

Pierson, W.R. and Brachaczek, W.W. (1974), "Airborne Particulate Debris from Rubber Tires", Rubber Chem. Technol., 47(5), 1275-99.

Pierson, W.R. and Brachaczek, W.W. (1975), "Airborne Particulate Debris from Rubber Tires", Proc. Environmental Aspects of Chemical Use in Rubber Processing Operations, EPA-560/1-75-002, March 12-14.

Porter, P.S. and Sommer, R.G. (1967), "Contact Dermatitis Due to Spandex", Arch. Derm., 95, 43-44.

Powell, R.S. (1969), "Thiazole Dyes", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 20, 185.

Prajapati, S.N., Soni, K.P. and Trivedi, A.M. (1972), "2-Mercaptobenzothiazole as a Corrosion Inhibitor for Copper, Brass and Aluminum in Halogen Substituted Acetic Acid", Indian J. Technol., 10(2), 73-5.

Rappaport, S.M. (1975), "The Identification of Effluents from Rubber Vulcanization", Proc. Environmental Aspects of Chemical Use in Rubber Processing Operations, EPA-560/1-75-002, March 12-14.

Revazova, Y.A. (1968), "Mutagenic and Morphogenic Activity of Some Rubber Mixture Ingredients (Preliminary Study)", Toksikol. Nov. Khim. Veshchestv, Vnedryaemykh Rezin. Shinnuyu Prom., 196-203.

Roberts, J.D. and Caserio, M.C. (1965), Basic Principles of Organic Chemistry, W.A. Benjamin Inc., New York City.

Rowe, S. (1969), "Primary Irritation Evaluation of Mildew Inhibitors G-4 and Vancide 512", U.S. Nat'l. Tech. Inform. Serv., AD-699810.

Schneider, G.B. (1974), "Proliferative Response to the Skin-Sensitizing Contact Agent Dinitrofluorobenzene in Lymph Nodes in Mice", Am. J. Anat., 141, 219-234.

Shaffer, C.B. (1971a), "Material Safety Data Sheet #8 - MBTS", American Cyanamid Co., Nov., 1971.

Shaffer, C.B. (1971b), "Material Safety Data Sheet #21 - CBS", American Cyanamid Co., Nov., 1971.

Shaffer, C.B. (1971c), "Material Safety Data Sheet #15 - ZMBT", American Cyanamid Co., Nov., 1971.

Shaffer, C.B. (1971d), "Material Safety Data Sheet #9 - 2-Mercaptobenzothiazole", American Cyanamid Co., Nov., 1971.

Shaffer, C.B. (1972a), "Material Safety Data Sheet #97 - TBBS", American Cyanamid Co., Jan., 1972.

Shaffer, C.B. (1972b), "Material Safety Data Sheet #96 - DIBS", American Cyanamid Co., Jan., 1972.

Shaffer, C.B. (1972c), "Material Safety Data Sheet #255 - MBT", American Cyanamid Co.

Shaffer, C.B. (1974), "Material Safety Data Sheet #12 - NOBS", American Cyanamid Co., June, 1974.

Shaver, W. (1968), "Rubber Chemicals", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 17, 509-542.

Shimidzu, N. and Uno, T. (1973), "Benzothiazole Derivatives as Chelating Agents. I. Syntheses of Benzothiazole Derivatives and Their Reactions with Metal Ions", Chem. Pharm. Bull., 21(1), 184-90.

Singh, I. and Banerjee, T. (1972), "Effect of 2-Mercaptobenzothiazole on Acid Dissolution and Hydrogen Absorption of Steel", Corros. Sci., 12(6), 503-515.

Squires, A.T.P.B. (1958), "Corrosion Inhibitors for Ethylene Glycol-Water for Piston Engines", Soc. Chem. Ind. (London) Monograph, 4, 51-68.

SRI (1972a), "Organic Rubber-Processing Chemicals - Salient Statistics", Chemical Economics Handbook, Stanford Research Institute, Menlo Park, California.

SRI (1972b), "Polychloroprene (Neoprene) Elastomers - Salient Statistics", Chemical Economics Handbook, Stanford Research Institute, Menlo Park, California.

SRI (1975), 1975 Directory of Chemical Producers, Stanford Research Institute, Menlo Park, California.

Stebletsova, V.D. and Evstifeev, M.M. (1971), "Extraction-Photometric Determination of Captax and Altax in Waste Waters", Metody Khim. Anal., Stokov Vod Predpr. Khim. Prom., 33-41.

Sullivan, F.A.V. (1956), "N-Oxydiethylene Benzothiazolesulfenamide, a Rubber Accelerator", U.S. Patent No. 2,758,995, August 14.

Thyasen, A.C., Bunker, H.J. and Adams, M.E. (1945), "Rubber Acid Damage in Fire Hoses", Nature, 155, 322-325.

Turk, S.D. (1969), "Thiols", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 20, 215.

Turner, N.J. (1966), "Industrial Fungicides", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 10, 232.

Uniroyal Chemical (1971a), "Product Safety Data Sheet - N-tert-Butyl-2-benzothiazole sulfenamide", June 29.

Uniroyal Chemical (1971b), "Product Safety Data Sheet - N-Cyclohexyl-2-benzothiazole sulfenamide", Sept. 29.

Uniroyal Chemical (1971c), "Product Safety Data Sheet - 2,2'-Dithiobisbenzothiazole", Sept. 29.

Uniroyal Chemical (1971d), "Product Safety Data Sheet - Zinc 2-Mercaptobenzothiazole", Nov. 11.

Uniroyal Chemical (1972), "Naugatuck Technical Data Sheets: Delac S, Delac NS, Delac MOR, MBT, MBTS, OXAF.

U.S. EPA (1975), "Organic Compounds Identified in Drinking Water", June 1 and September 1, Water Supply Research Laboratory, EPA, Cincinnati, Ohio.

U.S.I.T.C. (1959-1973), "Synthetic Organic Chemicals. U.S. Production and Sales", U.S. International Trade Commission, Washington, D.C. (1974p - preliminary data).

U.S. Tariff Commission (1974), "Imports of Benzenoid Chemicals and Products, 1973", TC Publication 688.

Vaisman, Y.I., Zaitseva, N.V. and Mikhailov, A.V. (1973), "Hygienic Standardization of the Components of Waste Water from Rubber Production in Reservoir Water", Gig. Sanit., (1973)(2), 17-21.

Vanderbilt Co., R.T. (1974), "Technical Data Sheet - NACAP", Petroleum Dep't., (NCP-1), Norwalk, CT.

Vanderbilt Co., R.T. (1975a), "Material Safety Data Sheet - Benzothiazyl Disulfide", Norwalk, CT.

Vanderbilt Co., R.T. (1975b), "Material Safety Data Sheet - 2-Mercaptobenzothiazole", Norwalk, CT.

Vanderbilt Co., R.T. (1975c), "Material Safety Data Sheet - Sodium 2-Mercaptobenzothiazole", Norwalk, CT.

Vanderbilt Co., R.T. (1975d), "Material Safety Data Sheet - Zinc 2-Mercaptobenzothiazole", Norwalk, CT.

Vanderbilt Co., R.T. (1975e), "Material Safety Data Sheet - 4-Morpholinyl-2-benzothiazole disulfide", Norwalk, CT.

Vanderbilt Co., R.T. (1975f), "Material Safety Data Sheet - N-Oxydiethylenebenzothiazole-2-sulfenamide", Norwalk, CT.

VanLare, E.J. (1965), "Cyanine Dyes", Kirk-Othmer Encycl. Chem. Technol., 2nd Ed., 6, 605.

Vorob'eva, R.S. and Mezentseva, N.V. (1962), "Toxicological Evaluation of Some Sulfenamide Derivatives of 2-Mercaptobenzothiazole Used as Accelerators in Vulcanization", Kauchuk i Rezina, 21(6), 14-16.

Vorob'eva, R.S. and Mezentseva, N.V. (1963), "Toxicological Evaluation of the Vulcanization Accelerator N,N-Diisopropyl-2-benzothiazolesulfenamide (Dipak) and of the Vulcanizing Agent p-Quinone Dioxime", Kauchuk i Rezina, 22(5), 27-8.

Vorob'eva, R.S. (1968), "N,N-Diisopropyl-2-benzothiazolesulfenamide", Toksikol. Nov. Khim Veshchestv, Vnedryaemykh Rezin. Shinnuyu Prom., 77-83.

Vorob'eva, R.S. and Mezentseva, N.V. (1968), "Sulfenamides Ts and M (Santocure and Santocure-MOR)", Toksikol. Nov. Khim. Veshchestv, Vnedryaemykh Rezin. Shinnuyu Prom., 64-71.

Vorob'eva, R.S., Kasparov, A.A. and Mezentseva, N.V. (1968), "2-Mercaptobenzothiazole", Toksikol. Nov. Khim. Veshchestv, Vnedryaemykh Rezin. Shinnuyu Prom., 28-35.

Walker, R.S. (1970), Building a Rubber Compound, R.T. Vanderbilt Co., Inc., New York.

Wang, C.Y. and Mellenthin, W.M. (1974), "Inhibition of Friction Discoloration on d'Anjou Pears by 2-Mercaptobenzothiazole", Hort Science, 9(3, Sec. 1), 196.

Wattenberg, L.W., Page, M.A. and Leong, J.L. (1968), "Induction of Increased Benzopyrene Hydroxylase Activity by 2-Phenylbenzothiazoles and Related Compounds", Cancer Res., 28(12), 2539-44.

Webb, R.G., Garrison, A.W., Keith, L.H. and McGuire, J.M. (1973), "Current Practice in GC-MS Analysis of Organics in Water", U.S. Nat'l. Tech. Inform. Serv., PB 224-947.

Weibull, B. (1962), "Reduction of 2-Mercaptobenzothiazole to Benzothiazole", Acta Chem. Scand., 16, 1052.

Weibull, B.J.G. (1966), "Behavior of Four Ethylene Glycol Antifreezes in Car Tests", Brit. Corrosion J., 1(7), 255-263.

Wilson, R.H., Planck, E.H. and McCormick, W.E. (1959), "Allergy in the Rubber Industry", Ind. Med. Surg., 28, 209-211.

Wilson, H.T.H. (1969), "Rubber Dermatitis: An Investigation of 106 Cases of Contact Dermatitis Caused by Rubber", Brit. J. Dermatol., 81, 175-179.

Winspear, G.G. (1958), Vanderbilt Rubber Handbook, R.T. Vanderbilt Co., Norwalk, CT, p. 367-375.

Woodroffe, G.L. and Munro, J.D. (1970), "Determination of Sodium Mercaptobenzothiazole and Benzotriazole in Inhibited Glycol Products", Analyst (London), 95(1127), 153-7.

Zaeva, G.N., Fedorova, V.I. and Fedechkina, N.I. (1966), "Estimation of the Toxicity of the Sulfenamides BT, M, and Ts", Toksikol. Novykh Prom. Khim. Veshchestv, 8, 70-83.